IUCLID

Data Set

Existing Chemical	:	ID: 92-52-4
CAS No.	:	92-52-4
EINECS Name	:	biphenyl
EC No.	:	202-163-5
TSCA Name	:	1,1'-Biphenyl
Molecular Formula	:	C12H10

Producer related part

Company	:	Dow Chemical, TERC
Creation date	:	04.02.2005

Substance related part

Company	:	Dow Chemical, TERC
Creation date	:	04.02.2005
Status	:	
Memo	:	
Printing date	:	02.05.2005
Revision date	:	
Date of last update	:	02.05.2005
Number of pages	:	113
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	SOCMA Biphenyl Working Group
Contact person	:	John Murray
Date	:	
Street	:	SOCMA
Town	:	
Country	:	
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

Source : Toxicology and Regulatory Affairs Freeburg, IL
24.12.2003

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.13 REVIEWS

2.1 MELTING POINT

Value : = 69 - 71 °C

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance :
Biphenyl, CASNO 92-52-
Reliability : (2) valid with restrictions

Handbook data are assigned reliability of
Flag : Critical study for SIDS endpoint
30.10.2003 (1)

2.2 BOILING POINT

Value : = 254 - 255 °C at 1010 hPa

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance :
Biphenyl, CASNO 92-52-
Reliability : (2) valid with restrictions

Handbook data are assigned reliability of
Flag : Critical study for SIDS endpoint
30.10.2003 (1)

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = .0119 hPa at 25 °C

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance :
Biphenyl, CASNO 92-52-
Reliability : (2) valid with restrictions

Published data are assigned reliability of
Flag : Critical study for SIDS endpoint
30.10.2003 (2)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = 4.01 at 25 °C
pH value :

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance :

Reliability	:	Biphenyl, CASNO 92-52- (2) valid with restrictions
Flag	:	Published data are assigned reliability of Critical study for SIDS endpoint

30.10.2003 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 7.28 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Remark	:	This result is supported by a second experimental value found in the EPIWIN 3.05 database as: Experimental Water Solubility Database Match: Name : BIPHENYL CAS Num : 000092-52-4 Exp WSol : 6.94 mg/L (25 deg C) Exp Ref : PEARLMAN,RS ET AL. (1984) This value is also supported by a measured value of 7.3 mg/L at 24.6 C reported by RD Wauchope and FW Getzen, Temperature Dependence of Solubilities and Heats of Fusion of Solid Aromatic Compounds. J Chem Eng Data 17:38 (1977
Source	:	Toxicology and Regulatory Affairs Freeburg, IL
Test substance	:	Biphenyl, CASNO 92-52-
Reliability	:	(2) valid with restrictions
Flag	:	Handbook data are assigned reliability of Critical study for SIDS endpoint

06.11.2003 (4)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	= .0000000000072 cm³/(molecule*sec)
Degradation	:	ca. 50 % after 18 hour(s)

Method :

INDIRECT PHOTOLYSIS:

Initial estimate based on AOP program in EPIWIN. The results of this calculation are shown below. There was also a match in the experimental reaction rate data base and this esperimental rate constant is shown below. There is a good correlation between the estimated reaction-rate constant and the experimental value.

AOP Program (v1.90) Results:
==========
SMILES : c1ccccc1c2ccccc2
CHEM : Biphenyl
MOL FOR: C12 H10
MOL WT : 154.21
---------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ----------

Hydrogen Abstraction	=	0.0000 E-12 cm3/molecule-sec
Reaction with N, S and -OH	=	0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds	=	0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds	=	0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings	=	6.7747 E-12 cm3/molecule-sec
Addition to Fused Rings	=	0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 6.7747 E-12 cm3/molecule-sec
HALF-LIFE = 1.579 Days (12-hr day; 1.5E6 OH/cm3)
HALF-LIFE = 18.946 Hrs
---------- SUMMARY (AOP v1.90): OZONE REACTION ----------

****** NO OZONE REACTION ESTIMATION ******
(ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
Chem Name : Biphenyl
CAS Number: 000092-52-4
Exper OH rate constant : 7.2 E-12 cm3/molecule-sec
Exper OH Reference: ATKINSON,R (1989)
Exper Ozone rate constant: < 2.0 E-19 cm3/molecule-sec
Exper NO3 rate constant : --- cm3/molecule-s

DIRECT PHOTOLYSIS: Biphenyl shows very little absorption of light at wavelengths greater than 290 nm, therefore, direct photolysis of the compound in air is unlikely to be an important process*

* (Moore WM et al; Soil Phase Photodegradation of Toxic Organics at Contaminated Disposal Sites for Soil Renovation and Groundwater Quality Protection. USGS Report No. G-1304, Reston, VA. NTIS PB-89-237267, Springfield, VA (1989) as cited in National Library of Medicine Hazardous Substance Data Base, Last Revision Date: 20020806

Result :

Direct: No photolysis expected

Indirect: Estimated half-life is ca 18 hours based on the calculated or the experimentally determined hydroxyl radical rate constant with Bipheny

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test substance :

Biphenyl, CASNO 92-52-

Reliability : (2) valid with restrictions

Estimate based on reliable reaction rate constant

Flag : Critical study for SIDS endpoint

13.12.2003 (5)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : > 1 year at 25 °C
t1/2 pH9 : at °C
Deg. product :
Method : other: estimated on chemical principles
Year :
GLP :
Test substance :

Method :

Estimate using chemical principle

Result :

Molecule does not contain a water-reactive or hydrolysable group. The following are considered water stable for this reason:

-Benzenes
-Biphenyls
-.....

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test substance :

Biphenyl, CASNO 92-52-

Conclusion :

Stable in water indefinitel

Reliability : (2) valid with restrictions

Estimate based on valid chemical principles and from EPIWIN are assigned a reliability of

Flag : Critical study for SIDS endpoint

30.10.2003 (6)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media : other: air-water-soil-sediment
Method : Calculation according Mackay, Level III
Year :

Method :

Measured values for physical values of Biphenyl were input into EPIWIN as shown below. Default biodegradation rates were determined to be in reasonable accord with experimental values. Model was allowed to assume equal distributions to air, water and soil. EQC Level model (as found in EPIWIN 3.05) was utilized

Result :

Results of the Level III fugacity modeling are:

Level III Fugacity Model (Full-Output):
==========

Chem Name : Biphenyl
Molecular Wt: 154.21
Henry's LC : 0.000308 atm-m3/mole (Henry database)
Vapor Press : 0.0089 mm Hg (user-entered)
Liquid VP : 0.0248 mm Hg (super-cooled)
Melting Pt : 70 deg C (user-entered)
Log Kow : 4.01 (user-entered)
Soil Koc : 4.2e+003 (calc by model)

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	5.54	35.7	1000
Water	28.8	360	1000
Soil	63.8	360	1000
Sediment	1.91	1440	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	7.09e-011	870	448	29	14.9
Water	2.31e-009	448	233	14.9	7.76
Soil	5.67e-010	993	0	33.1	0
Sediment	7.59e-010	7.44	0.309	0.248	0.0103

Persistence Time: 270 hr
Reaction Time: 349 hr
Advection Time: 1.19e+003 hr
Percent Reacted: 77.3
Percent Advected: 22.7

Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
Air: 35.66
Water: 360
Soil: 360
Sediment: 1440
Biowin estimate: 2.902 (weeks)

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+00

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test substance :

Biphenyl, CASNO 92-52-

Conclusion :

Under conditions of equal initial distribution to water, soil and air, Biphenyl is expected to distribute preferentially in soil > water > air > sediment

Reliability : (2) valid with restrictions

Estimate based on valid chemical principles and from EPIWIN are assigned a reliability of

Flag : Critical study for SIDS endpoint

24.12.2003 (7)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic

Inoculum : other: Natural river water

Concentration : 1 µg/l related to Test substance
100 µg/l related to Test substance

Contact time : 16 day(s)

Degradation : > 80 - 95 (±) % after 8 day(s)

Result :

Method :

Test water was collected from the Titabawassee river in Michigan upstream of any significant industrial or municipal discharge, filtered through course filter paper and used for testing within four hours of collection. Test material was dissolved directly in river water without use of a carrier by evaporating a hexane solution of Biphenyl on the inside surface of a glass jar and adding river water to the jar and rolling the jar to dissolve the test material. Serial dilutions of this stock were made to achieve the lower concentrations.

Two methods were used to estimate biodegradation. Hplc analysis of methylene chloride extracts of the incubation mixtures and evolution of carbon dioxide. Cabon-14 radiolabeled Biphenyl was utilized for the carbon dioxide evolution studies and carbon dioxide was trapped in ethanolamine and 2-methoxyethanol and determined by liquid scintillation counting.

The bacteria population of the river water was estimated using Millipore Total Count paddles incubated for three days before counting. An average of 6900 CFU/mL was determined from water collected on March 10, 1980 and used for some of the biodegradation studies

Result :

Concentrations of 1, 10 or 100 micrograms (ug)/L of Biphenyl were tested with freshly-collected river water on one day. Carbon dioxide evolution was rapid with estimated 50% evolution of carbon dioxide occurring after 1.5, 2 and 3 days of incubation in the dark at 20 C. This was confirmed using water collected on another day, which gave a 2.5-day 50% evolution of total carbon at the 1-ug/L concentration of Biphenyl.

Carbon dioxide evolution was measured from the Biphenyl degradation studies on days 1, 2, 3, 4, 8, and 16. Results of Biphenyl degradation are presented graphically in the publication and show 60% or greater carbon dioxide evolution at 4-day sampling time for concentrations of 1, 10 and 100 ug/L. The best recovery was obtained at 100 ug/L (where the bacteria take up a smaller percentage of the organic carbon) where 80% evolution was obtained at 8 days and ca 97% at 16 days. Carbon dioxide evolution curves were similar at the lower concentrations of test substance but recovery of carbon dioxide was only about 80 % at 1 and 10 ug/L. At all concentrations, the biodegradation appeared to be complete by 16 days after start.

Parent compound was also monitored by HPLC after methylene chloride extraction. Loss of Biphenyl was rapid, showed no induction period, and was essentially complete (less than ca 5% remaining) after 4 days of incubation. Identification of a metabolite at about the 2 to 5% level of parent was made but the metabolite was not identified.

Material balance studies were conducted to determine the total recover of radiolabeled carbon by measuring the amount of radioactivity remaining in the river water after carbon dioxide evolution and extraction of parent material and metabolites with methylene chloride. Individual material balances are not given but it was determined that the mean accountability of carbon-14 for all studies conducted in this publication (including studies with chlorinated biphenyls) was 92.2%. It was determined that the typical loss of 5% of the radiocarbon came from purging of equipment during carbon dioxide measurements and volatilization during setu

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test substance :

Biphenyl, CASNO 92-52-

Conclusion :

Biphenyl is rapidly biodegraded to carbon dioxide in typical river water from an area that drains primarily agricultural activities and is upstream from major industrial or municipal effluents. The half-life in river water is on the order of 2 days

Reliability : (1) valid without restriction

A carefully conducted and well-documented publication from a GLP study

Flag : Critical study for SIDS endpoint

13.12.2003 (8)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	192 hour(s)
Unit	:	mg/l
NOEC	:	= .17 measured/nominal
LC50	:	= 1.3 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	other: conducted according to a Test Rule 40 CFR Part 799 Federal Register Vol 50#177 Thursday 12 Sept. 1985.
Year	:	1985
GLP	:	yes
Test substance	:	

Method :

Animals: Rainbow trout (Salmo gairdneri Richardson) used in acute testing were obtained as eyed embryos from Mt. Lassen Trout Farms, Red Bluff California on March 11, 1987. Upon arrival they were placed in a trout hatcher and incubated at 12 ± 2°C until hatched. Juveniles were held in 110 L stainless steel aquaria at a water temperature of 12 ± 2°C, and were provided a 16-h light/8-h dark photocycle. A synthetic diet was provided ad libitum. Juvenile trout ca. 160 days post-hatch were acclimated to test temperature at least 72-h prior to testing.

The 192-hour flow-through acute test was conducted with juvenile rainbow trout. There were six test concentrations, an acetone control with the acetone concentration equaling the highest concentration in any treatment group (0.1 ml/L) and a water control. Each test concentration and control was set in duplicate with each replicate containing 10 fish. During each cycle of the diluter, 1 L of test solution or water was delivered to each replicate.

Water: The water supply is pumped from the Upper Saginaw Bay of Lake Huron. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. As it enters the laboratory, the water is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the test; pH 7.5 to 7.8 hardness (mg/L as calcium carbonate) 73 to 76 alkalinity (mg/L as calcium carbonate) 48 to 54 and conductivity 150 to 160 (umhos/cm).

Dilutor: An intermittent-flow proportional diluter system was used. This system was designed to deliver six test concentrations, a carrier and water control. The diluter was calibrated so that the concentration of the test material in each treatment below the high concentration was approximately 65 percent of that in the next higher treatment level. The carrier control received acetone at a concentration equaling the highest concentration in any treatment group, (no more than 0.1 ml/L). The diluter operates as follows: a precision dosing system delivers the test material from a stock bottle to a mixing chamber where it is mixed with dilution water and then distributed to "toxicant cells". When the diluter cycles, the test material from each toxicant cell blends with water from its respective dilution water cell and then flows into mixing/splitting chambers. Silicone delivery tubes from these chambers provide approximately 500 mL to the test aquaria, which are positioned on one tier, side by side, in a temperature-controlled water trough.
The diluter was calibrated prior to the beginning of the tests and was found to be operating normally. The diluter was set to provide at least 15 volumes turnovers in the test aquaria each twenty-four hours.

The test vessels were constructed of double-strength glass glued with clear silicone adhesive, and measure approximately 30 x 15 x 14 cm deep. Each is provided with a nylon screen covered drain that maintains a water volume of 3.7 liters. In the embryo-larval test, the embryos were incubated in circular (124 mm in diameter by 51 mm high) cups with 360 um nylon screen bottoms that were supported in the test vessels by glass beads. The flow from the delivery tube was directed into the incubation cup to produce a flow of water around the embryos during the incubation period.

Statistics: The flow-through acute concentration-mortality data were analyzed for daily LC50 values. A computer program was used to calculate the LC50 values and corresponding 95% confidence intervals. (Stephan, U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota). This program has three methods available; probit analysis, moving average angle analysis, and binomial probability

Result : Analyzed biphenyl concentration ranged between 83 and 110 percent of nominal. The 192-hour LC50 based on average measured concentrations, was determined to be 1.3 mg/L. The 24 through 120-h LC50 values were not determined due to insufficient mortality. Sublethal effects such as complete loss of equilibrium (immobile on bottom) or partial loss of equilibrium (the inability to maintain normal swimming posture) were observed at 0.81 mg/L and higher. Anorexia was observed at 0.60 mg/L and higher; melanosis and long fecal casts were observed at 0.27 mg/L and higher.

Anal		PERCENT DEAD AT (hours)							
Conc	No	24	48	72	96	120	144	168	192
(mg/L)	Fish	--	--	--	--	---	---	---	---
1.502*	20	10	10	15	40	45	60	65	70
0.812*	20	0	0	0	0	0	0	0	0
0.604*	20	0	0	0	0	0	0	0	0
0.373*	20	0	0	0	0	0	0	0	0
0.272*	20	0	0	0	0	0	0	0	0
0.171	20	0	0	0	0	0	0	0	0
0.000	20	0	0	0	0	0	0	0	0

* = Sublethal effects

The control data as presented is applicable to both the acetone control group and the water control group.

Analytically determined concentrations

Day 0 (mg/L)	Day 3 (mg/L)	Day 8 (mg/L)	Mean ± S. D. (n = 4 or 5)
1.593	1.305	1.381	1.502 ± 0.15
0.886	0.830	0.743	0.812 ± 0.061
0.595	0.592	0.623	0.604 ± 0.029
0.403	0.361	0.380	0.373 ± 0.024
0.282	0.259	0.280	0.272 ± 0.011
0.171	0.154	0.174	0.171 ± 0.01

Composite samples of both replicates were also measured on Days 2 and 5. These data points were used to calculate the Mean and SD presented above but are not included here.

Test condition : ----------CONDITIONS----------

Temperature	12 ± 1°C
Photoperiod	16 hrs light/8 dark
Aeration	None
Type of Test	Acute
Diet	None 1st 96 hours; once daily thereafter
Test Vessel Size	Approximately 30x15x14 cm deep

	Test Volume	3.7 L
	No. of Treatment groups	6
	No. of Replicates/Treatment	2
	Organisms/Replicate	10
	Observations	D.O., pH, temperature morality, sublethal effects
	Effect Criteria	Sublethal effects and mortality
	Length of Test	192 hours
	Mean wt of fish	0.647 g
	Dissolved Oxygen	>83% Saturation (7.7 - 9.0 mg/L

Test substance : Biphenyl, CASNO 92-52-4
Purity of test material was >99.1%.

Conclusion : The 192-hour LC50 for biphenyl under these conditions is 1.36 (0.81-1.5) mg/L
The 192-hour NOEC for biphenyl under these conditions is 0.17 mg/L

Sublethal effects were noted at 0.27 mg/L and higher during the tes

Reliability : (1) valid without restriction
High quality guideline-like study under GLP with analytical suppor

Flag : Critical study for SIDS endpoint
15.02.2005 (9)

Type : static
Species : Cyprinodon variegatus (Fish, estuary, marine)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 4.6 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: EPA-660/3-75-009
Year : 1975
GLP : yes
Test substance :

Method : Static acute toxicity tests were conducted using standard test methods (EPA-660/3-75-009). Briefly, groups of ten fish were exposed to nominal concentrations of biphenyl in 10 liters of water. A total of seven exposure concentrations between 3.6 and 8.2 mg/L (3.6, 4.4, 5.2, 5.9, 6.6, 7.4 and 8.2 mg/L) were conducted. In addition, a control group consisting of 10 fish were exposed to carbon filtered Lake Huron water containing no more than 0.5 ml/L acetone since it was used as a carrier solvent. Stock solutions were prepared using acetone as the carrier solvent. The carrier solvent concentration did not exceed 0.5 ml/L of dilution water.

Fish were allowed to acclimate to the test aquaria in eight liters of dilution water for 24 hours with aeration prior to the addition of the toxicant. Aeration was stopped at the start of the test. The fish were not fed during the acclimation period nor the 96-hr exposure period.

A 16-hour light/8-hour dark photoperiod was provided with illumination provided at 1500/1725 lux by cool white fluorescent bulbs. The water temperature was maintained at 12C. Death, defined as no response to a gentle prodding and no gill cover movement, was used as the effect criterion. The fish were observed, and dead fish were removed at 24-hour intervals.

Seawater obtained from the Gulf of Mexico was used. This water is passed sequentially through sand, Cuno cartridge filters, and a diatomaceous earth final filter. It has the following typical characteristics: pH 8; Salinity 2%; total organic carbon 3 mg/L; total suspended solids 3 mg/L; and conductivity 30,500 uS/cm.

Sheepshead minnows (Cyprinodon variegatus) were obtained from Sea Plantations, Salem Massachusetts. They were held in an 812 L fiberglass tub for at least ten days prior to testing. Holding conditions were as follows: fluorescent illumination; photoperiod, 16 hour light/8 hour dark; and temperature, 22C. They were fed Tetramin Staple Food at least once daily.

Statistical Calculations: The results from the aquatic organism toxicity tests are reported as the LC50 value. The nominal toxicant concentrations of each aquaria were used to calculate the LC50 values. LC50 values were calculated using a computer program of Finney's method of probit analysis and Thompson's method of moving averages. The computer program was supplied by C. Stephan, US EPA, Duluth, MN. The probit results are generally considered the most appropriate and are reported when possible. In tests where the data did not fit the probit program, the moving average LC50's are reported.

According to the Committee on Methods for Toxicity Tests with Aquatic Organisms, a valid toxicity test must have one test concentration which killed or affected less than 35% of the fish exposed to it, and one test concentration which killed or affected more than 65% of the fish.

Result : The 96 hour LC50 was 4.6 mg/L (95% CI 4.0-5.0 mg/L) (Table 1).

Table 1

Conc. mg/L	Number Exposed	Number Dead
0	10	0
3.6	10	2
4.4	10	4
5.2	10	6
5.9	9	8
6.6	10	10
7.4	10	10
8.2	10	10

Test substance : Custom synthesized biphenyl (lot OCR 564:86) was prepared by Dow Chemical Company. Test material was analyzed by gas chromatography-thermal conductivity and infrared spectroscopy. There were no impurities identified at a limit of detection of 0.02%.

08.02.2005 (10) (11)

Type : static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 4.7 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: EPA-660/3-75/009
Year : 1975
GLP : yes
Test substance :

Method : Static acute toxicity tests were conducted using standard test methods (EPA-660/3-75-009). Briefly, groups of ten fish were exposed to nominal concentrations of biphenyl in 10 liters of water. A total of ten exposure concentrations between 2.1 and 7.5 mg/L were conducted. In addition, a control group consisting of 10 fish were exposed to carbon filtered Lake Huron water containing no more than 0.5 ml/L acetone since it was used as a carrier solvent. Stock solutions were prepared using acetone as the carrier solvent. The carrier solvent concentration did not exceed 0.5 ml/L of dilution water.

Fish were allowed to acclimate to the test aquaria in eight liters of dilution water for 24 hours with aeration prior to the addition of the toxicant. Aeration was stopped at the start of the test. The fish were not fed during the acclimation period nor the 96-hr exposure period.

A 16-hour light/8-hour dark photoperiod was provided with illumination provided at 1500/1725 lux by cool white fluorescent bulbs. The water temperature was maintained at 12C. Death, defined as no response to a gentle prodding and no gill cover movement, was used as the effect criterion. The fish were observed, and dead fish were removed at 24-hour intervals.

Carbon-filtered Lake Huron water was used in all freshwater tests. It typically had the following characteristics: conductivity, 184 uS/cm; hardness [as calcium carbonate (CaCO3)], 107 mg/L; alkalinitiy (as CaCO3), 82 mg/L and pH, 7.8.

Bluegill (Lepomis macrochirus) were obtained from Osage Catfisheries, Osage Beach, Missouri. They were held in an 800 L fiberglass tub for at least ten days prior to testing. Holding conditions were as follows: flow rate, 2 L/min; illumination, 430-646 lux; photoperiod, 16 hour light/8 hour dark; and temperature, 22C +/- 1C. They were fed a synthetic diet at least once daily.

Statistical Calculations: The results from the aquatic organism toxicity tests are reported as the LC50 value. The nominal toxicant concentrations of each aquaria were used to calculate the LC50 values. LC50 values were calculated using a computer program of Finney's method of probit analysis and Thompson's method of moving averages. The computer program was supplied by C. Stephan, US EPA, Duluth, MN. The probit results are generally considered the most appropriate and are reported when possible. In tests where the data did not fit the probit program, the moving average LC50's are reported.

According to the Committee on Methods for Toxicity Tests with Aquatic Organisms, a valid toxicity test must have one test concentration which killed or affected less than 35% of the fish exposed to it, and one test concentration which killed or affected more than 65% of the fish.

Result : The 96 hour LC50 was 4.7 mg/L (95% CI 4.3-5.1 mg/L) (Table 1).

Table 1

Conc. mg/L	Number Exposed	Number Dead
0	10	0
2.1	10	0
2.4	10	0
2.8	10	0
3.2	10	0
3.7	10	2
4.2	10	1
4.9	10	5
5.6	10	10
6.5	10	10
7.5	10	10

Test substance : Custom synthesized biphenyl (lot OCR 564:86) was prepared by Dow Chemical Company. Test material was analyzed by gas chromatography-thermal conductivity and infrared spectroscopy. There were no impurities identified at a limit of detection of 0.02%.

5. Toxicity

Id 92-52-4
Date

Type	:	static
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 1.5 measured/nominal
Limit test	:	
Analytical monitoring	:	no
Method	:	other: EPA-660/3-75-009
Year	:	1975
GLP	:	yes
Test substance	:	

Method :

Static acute toxicity tests were conducted using standard test methods (EPA-660/3-75-009). Briefly, groups of ten fish were exposed to nominal concentrations of biphenyl in 10 liters of water. A total of ten exposure concentrations between 0.18 and 1.8 mg/L were conducted. In addition, a control group consisting of 10 fish were exposed to carbon filtered Lake Huron water containing no more than 0.5 ml/L acetone since it was used as a carrier solvent. Stock solutions were prepared using acetone as the carrier solvent. The carrier solvent concentration did not exceed 0.5 ml/L of dilution water.

Fish were allowed to acclimate to the test aquaria in eight liters of dilution water for 24 hours with aeration prior to the addition of the toxicant. Aeration was stopped at the start of the test. The fish were not fed during the acclimation period nor the 96-hr exposure period.

A 16-hour light/8-hour dark photoperiod was provided with illumination provided at 1500/1725 lux by cool white fluorescent bulbs. The water temperature was maintained at 12C. Death, defined as no response to a gentle prodding and no gill cover movement, was used as the effect criterion. The fish were observed, and dead fish were removed at 24-hour intervals.

Carbon-filtered Lake Huron water was used in all freshwater tests. It typically had the following characteristics: conductivity, 184 uS/cm; hardness [as calcium carbonate (CaCO3)], 107 mg/L; alkalinitiy (as CaCO3), 82 mg/L and pH, 7.8.

Rainbow trout (Salmo gairdneri) were hatched from eyed eggs obtained from Mt Lassen Trout Farm, Red Bluff, CA. Eggs were ten days prehatch when received. They were hatched in a fish incubator which received one liter/minute of 12C dilution water. When the trout reached the 'swim-up' stage of development, they were transferred to 110 L stainless tanks and held under the following conditions for at least 3 weeks: flow rate, 1 L/min; illumination, 960-1150 lux; photoperiod, 16 hour light/8 hour dark; and temperature, 12C +/- 1C. They were fed Mastermix F45 Fish Starter (Central Soya and Subsidiaries, Fort Wayne, IN) ad libitum.

Statistical Calculations: The results from the aquatic organism toxicity tests are reported as the LC50 value. The nominal toxicant concentrations of each aquaria were used to calculate the LC50 values. LC50 values were calculated using a computer program of Finney's method of probit analysis and Thompson's method of moving averages. The computer program was supplied by C. Stephan, US EPA, Duluth, MN. The probit results are generally considered the most appropriate and are reported when possible. In tests where the data did not fit the probit program, the moving average LC50's are reported.

According to the Committee on Methods for Toxicity Tests with Aquatic Organisms, a valid toxicity test must have one test concentration which killed or affected less than 35% of the fish exposed to it, and one test

concentration which killed or affected more than 65% of the fish.

Result : The 96 hour LC50 was 1.5 mg/L (95% CI 1.4-1.6 mg/L).

Table 1

Conc. mg/L	Number Exposed	Number Dead
0.00	10	0
0.18	10	0
0.32	10	0
0.56	10	0
0.70	10	0
0.86	10	0
1.00	10	0
1.15	10	0
1.35	10	1
1.55	10	6
1.80	10	10

Test substance : Custom synthesized biphenyl (lot OCR 564:86) was prepared by Dow Chemical Company. Test material was analyzed by gas chromatography-thermal conductivity and infrared spectroscopy. There were no impurities identified at a limit of detection of 0.02%.

09.02.2005 (10) (11)

Type : static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : > 13 measured/nominal
Limit test :
Analytical monitoring : no
Method : other: EPA-660/3-75-009
Year : 1975
GLP : yes
Test substance :

Method : Static acute toxicity tests were conducted using standard test methods (EPA-660/3-75-009). Briefly, groups of ten fish were exposed to nominal concentrations of biphenyl in 10 liters of water. A total of ten exposure concentrations between 1 and 13 mg/L were conducted. In addition, a control group consisting of 10 fish were exposed to carbon filtered Lake Huron water containing no more than 0.5 ml/L acetone since it was used as a carrier solvent. Stock solutions were prepared using acetone as the carrier solvent. The carrier solvent concentration did not exceed 0.5 ml/L of dilution water.

Fish were allowed to acclimate to the test aquaria in eight liters of dilution water for 24 hours with aeration prior to the addition of the toxicant. Aeration was stopped at the start of the test. The fish were not fed during the acclimation period nor the 96-hr exposure period.

A 16-hour light/8-hour dark photoperiod was provided with illumination provided at 1500/1725 lux by cool white fluorescent bulbs. The water temperature was maintained at 12C. Death, defined as no response to a gentle prodding and no gill cover movement, was used as the effect criterion. The fish were observed, and dead fish were removed at 24-hour intervals.

Carbon-filtered Lake Huron water was used in all freshwater tests. It typically had the following characteristics: conductivity, 184 uS/cm; hardness [as calcium carbonate (CaCO3)], 107 mg/L; alkalinitiy (as CaCO3), 82 mg/L and pH, 7.8.

Fathead minnow (Pimephales promelas) were obtained from Osage Catfisheries, Osage Beach, Missouri. They were held in an 800 L fiberglass tub for at least ten days prior to testing. Holding conditions were as follows: flow rate, 2 L/min; illumination, 430-646 lux; photoperiod, 16 hour light/8 hour dark; and temperature, 12C +/- 1C. They were fed a synthetic diet at least once daily.

Statistical Calculations: The results from the aquatic organism toxicity tests are reported as the LC50 value. The nominal toxicant concentrations of each aquaria were used to calculate the LC50 values. LC50 values were calculated using a computer program of Finney's method of probit analysis and Thompson's method of moving averages. The computer program was supplied by C. Stephan, US EPA, Duluth, MN. The probit results are generally considered the most appropriate and are reported when possible. In tests where the data did not fit the probit program, the moving average LC50's are reported.

According to the Committee on Methods for Toxicity Tests with Aquatic Organisms, a valid toxicity test must have one test concentration which killed or affected less than 35% of the fish exposed to it, and one test concentration which killed or affected more than 65% of the fish.

Remark : This concentration is approximately 2 fold higher than the stated water solubility.

Result : The 96 hour LC50 was >13 mg/L. At the highest concentration tested, 13 mg/L, there was no mortality observed.

Test substance : Custom synthesized biphenyl (lot OCR 564:86) was prepared by Dow Chemical Company. Test material was analyzed by gas chromatography-thermal conductivity and infrared spectroscopy. There were no impurities identified at a limit of detection of 0.02%.

08.02.2005 (10) (11)

Remark : ECOSAR Program (v0.99f) Results:

==========

SMILES : c1ccccc1c2ccccc2

CHEM : Biphenyl

CAS Num:

ChemID1:

ChemID2:

ChemID3:

MOL FOR: C12 H10

MOL WT : 154.21

Log Kow: 4.01 (User entered)

Melt Pt: 70.00 deg C

Wat Sol: 7.48 mg/L (measured)

ECOSAR v0.99f Class(es) Found

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR	: Fish	14-day	LC50	3.676
(Baseline Toxicity)				
Neutral Organics	: Fish	96-hr	LC50	1.475
Neutral Organics	: Fish	14-day	LC50	3.676
Neutral Organics	: Daphnid	48-hr	LC50	1.816
Neutral Organics	: Green Algae	96-hr	EC50	1.274
Neutral Organics	: Fish	30-day	ChV	0.263
Neutral Organics	: Daphnid	16-day	EC50	0.224
Neutral Organics	: Green Algae	96-hr	ChV	0.407
Neutral Organics	: Fish (SW)	96-hr	LC50	0.893
Neutral Organics	: Mysid Shrimp	96-hr	LC50	0.101
Neutral Organics	: Earthworm	14-day	LC50	228.056 *

Reliability : (2) valid with restrictions
09.02.2005

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : flow through
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC0 : = .04 measured/nominal
EC50 : = .36 measured/nominal
EC100 : > .96 measured/nominal
Limit Test : no
Analytical monitoring : yes

5. Toxicity

Id 92-52-4
Date 02.05.2005

Method	:	other: conducted according to a Test Rule 40 CFR Part 799 Federal Register Vol 50#177 Thursday 12 Sept. 1985.
Year	:	1985
GLP	:	yes
Test substance	:	

Method :

Animals: Daphnia magna Straus, 1820, was used as the test organism in this study. The daphnids were cultured in the laboratory from parthenogenetic females. On the day before testing began, reproductively mature females were isolated. Young produced by these adults were collected and used for testing within 24 hrs.

The acute flow-through toxicity test consisted of exposing groups of 10 neonate daphnids to five concentrations of the test material, a carrier control (acetone 0.1 ml/L) and a water control. The five test concentrations and the controls were set in triplicate, resulting in 30 neonate daphnids being exposed to each concentration. The test vessels were maintained in a temperature controlled water trough set at 20±1°C. Dissolved oxygen, pH and temperature were measured in the high, middle, low and control concentrations daily. The duration of this test was 48 hrs.

The exact loading cannot be accurately determined from the description in the report. The "test vessel" is described as a 400 mL beaker, but it is not clear what quantity of test water was used. Assuming the beaker was notched and 75% full at overflow, the loading would have been 1 daphnid/30 mL.

Water: The water supply is pumped from the Upper Saginaw Bay of Lake Huron. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. As it enters he laboratory, the water is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the test; pH 7.4 to 7.7, hardness (mg/L as calcium carbonate) 73 to 78, alkalinity (mg/L as calcium carbonate) 49 to 52 and conductivity (umhos/cm) 160 to 170.

Dilutor: Testing was conducted with an intermittent-flow proportional diluter equipped with a Micromedic automatic pipette, which was triggered to inject the appropriate amount of test material into the toxicant mixing chamber at the beginning of each cycle. The toxicant mixing chamber was equipped with a recirculating pump that provided mixing for at least three minutes before the solution was delivered to the testing chambers. The diluter had a dilution factor of about 0.50. At each cycle 500 ml test solution or control water was delivered to each flow-splitting dilution chamber. These chambers, which were randomly positioned on the diluter, diverted ca. 125 ml to each of four replicate test chambers at each test concentration and the control during the acute test. During the chronic study, the 125 ml delivered from the Splitter cells to each replicate was split five ways into each of the five tubes contained within a replicate beaker. The diluter was set to cycle every 30 minutes resulting in a minimum of 15 volume replacements in each beaker per day.

Statistics: The LC50 and 95% confidence intervals were determined for the 48-hour acute test using probit analysis. The LC50 values were based on analyzed concentrations. The LC50 value is the statistically determined concentration of the test material at which 50% of the test organisms would die within a specified time interval

Remark : No specific recognized guideline is referenced in the report. The study was conducted in compliance with the TSCA Section 4 Test Rule for Biphenyl and the protocol was approved by the EPA prior to study initiation. The study followed the Dow SOP #ET-25-1987-1 "Daphnia magna flow-through acute toxicity test"

Result : The mean biphenyl concentrations derived from the analyzed test solutions during the acute test are shown in the table. All analyzed concentrations were within a range of 63.3 to 97.6% of nominal. The calculated 48-hr LC50 value for biphenyl was 0.36 mg/L (95% confidence interval: 0.28 to 0.47 mg/L). The no observable effect level was 0.04 mg/L and the 100 % kill concentration was > 0.96 mg/L. There was no mortality in the acetone controls and 1%, mortality in the water controls over the 48 h test period. No sublethal effects were observed during this test.- The dissolved oxygen (D.O.) measurements throughout the test were all >90% saturation. The pH and temperature measurements ranged from 7.4 to 7.9 and 20.5 to 20.7°C, respectively.

Analy Conc (mg/L)	No Daphnia	% Dead24-hr	% Dead48-hr
0.96	30	30	87
0.48	30	7	57
0.24	30	0	40
0.09	30	0	7
0.04	30	0	0
Acetone	30	0	0
Water	30	0	3

No sublethal effects were noted during the test

Test condition : Conditions

Temperature	20 ± 1°C
Photoperiod	16 hrs light/8 dark
Daphnid source	laboratory reared
Diet	NA
Test Vessel	400 mL beaker
Observations	D.O. pH,temperature, mortality 0, 24, 48 hrs
Effect Criteria	mortality-immobility
Length of Test	48 hrs

Analytically determined concentrations

Nominal Concen. (mg/L)	Day 0 (mg/L)	24 H (mg/L)	48 H (mg/L)	Mean ± S. D. (n = 6)	Percent Nominal
1	1.13	0.797	0.965	0.964 ±0.161	96.4
0.5	0.529	0.433	0.493	0.485 ±0.048	97
0.25	0.258	0.215	0.259	0.244 ±0.025	97.6
0.13	0.103	0.086	0.093	0.094 ±0.009	72.3
0.06	0.043	0.034	0.037	0.038 ±0.005	63.3
Acetone	N.D.	N.D.	N.D.	N.D.	
Water	N.D.	N.D.	N.D.	N.D.	

Water Quality Measurements

Temperature Range:	20.5 to 20.7 °C
pH Range:	7.4 to 7.9
Dissolved Oxygen:	> 90% saturat

Test substance : Biphenyl, purity > 99.2% CAS# 92-52-4

Conclusion : The 24-hour EC50 for biphenyl under these conditions is 1.3 mg/L (95% confidence limits of 1.0 - 3.9)

-The 24-hour NOEC for biphenyl under these conditions is 0.24 mg/L

-The 48-hour EC50 for biphenyl under these conditions is 0.36 mg/L (95% confidence limits of 0.28 - 0.47)

-The 48-hour NOEC for biphenyl under these conditions is 0.04 mg/L

No sublethal effects were noted during the te

Reliability : (1) valid without restriction
High quality guideline-like study under GLP with analytical suppor

Flag : Critical study for SIDS endpoint

15.02.2005 (12)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = .25 measured/nominal
EC50 : = .73 measured/nominal
Limit Test : no
Analytical monitoring : no
Method :
Year :
GLP : no
Test substance :

Method :

A static toxicity test was conducted using clear-capped glass jars (8 oz) containing about 200 mL test solution. The dilution water was local well water. The test substance, dissolved in dimethyformamide (DMF), was pipetted into 1000 ml of dilution water and shaken for 1 minute for each concentration. This solution was then divided into three 200 ml aliquots in triplicate jars. The remaining 400 mL were used for 0-hour DO, pH, alkalinity and hardness determinations. A control, consisting of the same dilution water and conditions but without test material was established. Also, a solvent control was employed which consisted of dilution water and the maximum amount of solvent used in the test concentrations (0.5 ml/L). Special attention was given to place polyethylene lined caps on all jars after the Daphnia were added to prevent any loss of Biphenyl due to volatilization. The caps were removed only once during the study to count the Daphnia at 24 hours

All test vessels were maintained at room temperature without aeration during the test. Ten daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 30 daphnids per concentration level and controls. During this test, the dissolved oxygen concentration, pH, alkalinity, hardness, conductivity and temperature of test solutions were monitored at the initiation in the control and high text concentration and termination of the toxicity test in the high, middle, low and control test concentrations.

Statistical methods:
Test concentrations and corresponding percent immobilization data derived from definitive tests were used to calculate the 48-hour median effect concentration, EC50, and 95% confidence intervals.
In tests where the highest percentage immobilization was > 65 percent, the computer program of Stephan, which calculates EC50 by three methods, binomial, moving average, and probit analysis, was used (Stephan). For tests in which the immobilization did not exceed 50 percent, the EC50 is reported as greater than the highest test concentration. If the highest percentage immobilization was >50 <65 percent, the EC50 is estimated by the program of Stephan and is reported as an estimate.

Stephan, C. E. 1976. Methods for Calculating an LC50. In Aquatic Toxicology and Hazard Evaluation, F. L. Mayer and J. L. Hamelink Editors

Remark :

The fact that it was stated in the report that the water solubility of the test material was exceeded at 2.0 mg/L and above is of concern. The published value for water solubility of biphenyl is 7.38 mg/L. It is possible the water conditions were such that the solubility was reduced; however, not considered likely based on the study reported in 1983 using essentialy the same conditions in which the test material was reported to be fully soluble up to 5 mg/

Result :

Visual inspection of the beakers indicated that the water solubility was exceeded at concentrations of 2.0 mg/L and higher. This should not effect the EC50 valuesince the key data points used to calculate the 48-hour EC50 were derived from exposure concentrations which were in solution.

Concentrations tested and corresponding percent immobilization of Daphnia magna exposed to Biphenyl.

PERCENT IMMOBILIZATION

CONC mg/L	24-Hrs	48-Hrs
Control	0	0
Solvent Control	0	0
0.25	0	3.3
0.50	0	13.3
1.0	3.3	76.6
2.0	0	100
4.0	26.6	100

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test condition :

During the 48-hour toxicity test with Biphenyl, the pH and dissolved oxygen ranged from.7.9 to 8.2 and 7.0 to 8.6 mg/L, respectively (Tables 2 and 3 and Appendix I). The average temperature was 22°C and the alkalinity and hardness ranged from 220 to 258 mg/L and 230 to 262 mg/L.

PARAMETER	CONC. (mg/L)	TIME 0-Hr	48-Hr
Temperature (°C)	Control	22	22
	0.25	22	22
	1	22	22
	4	22	22
D.O. (mg/L)	Control	7.5	8
	0.25	7	8.3
	1	7.9	8.7
	4	7.6	8.6
pH	Control	8	7.9
	0.25	8.2	8
	1	8.2	8
	4	8	7.9
Alkalinity (mg/L)	Control	232	238
	0.25	230	258
	1	242	220
	4	226	250

Hardness (mg/L)	Control	256	230
	0.25	274	250
	1	230	246
	4	262	236

Test substance :

Biphenyl, CASNO 92-52-

Conclusion :

Under these conditions, the 48-hour EC50 for Daphnia magna is 0.73 mg/L (confidence limits of 0.63 to 0.85) and the NOEC is 0.25 mg/L

Reliability : (2) valid with restrictions

Although this study was not conducted under full GLPs, it was conducted by a scientifically defensible method following a standard laboratory guideline it was stated that the water solubility of the test material was exceeded at 2.0 mg/L and above.

23.12.2003 (13)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = 1.8 measured/nominal
EC50 : = 3.65 measured/nominal
Limit Test : no
Analytical monitoring : no
Method :
Year :
GLP : no data
Test substance :

Method :

A static toxicity test was conducted using clear-capped glass jars (8 oz) containing about 200 mL test solution. The dilution water was local well water. The test substance, dissolved in dimethyformamide (DMF), was pipetted into 1000 ml of dilution water and shaken for 1 minute for each concentration. This solution was then divided into three 200 ml aliquots in triplicate jars. The remaining 400 mL were used for 0-hour DO, pH, alkalinity and hardness determinations. A control, consisting of the same dilution water and conditions but without test material was established. Also, a solvent control was employed which consisted of dilution water and the maximum amount of solvent used in the test concentrations (0.5 ml/L).

Nominal test concentrations were selected based on a rangefinding test. All test vessels were maintained at room temperature without aeration during the test. Ten daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 30 daphnids per concentration level and controls. During this test, the dissolved oxygen concentration, pH, alkalinity, hardness, conductivity and temperature of test solutions were monitored at the initiation in the control and high text concentration and termination of the toxicity test in the high, middle, low and control test concentrations.

Statistical methods:
Test concentrations and corresponding percent immobilization data derived from definitive tests were used to calculate the 48-hour median effect concentration, EC50, and 95% confidence intervals.
In tests where the highest percentage immobilization was > 65 percent, the computer program of Stephan, which calculates EC50 by three methods, binomial, moving average, and probit analysis, was used (Stephan). For tests in which the immobilization did not exceed 50 percent, the EC50 is reported as greater than the highest test concentration. If the highest

percentage immobilization was >50 <65 percent, the EC50 is estimated by the program of Stephan and is reported as an estimate.

Stephan, C. E. 1976. Methods for Calculating an LC50. In Aquatic Toxicology and Hazard Evaluation, F. L. Mayer and J. L. Hamelink Editors.

Result :

A summary of the percent immobilization during this study is presented in the table below.

Visual inspection of the beakers indicated that the water solubility was not exceeded at any concentration.

CONC	PERCENT IMMOBILIZATION	
mg/L	24-Hrs	48-Hrs
Control	0	0
Solvent Control	0	0
0.65	0	0
1.08	0	0
1.8	0	0
3	0	17
5	0	97

Concentrations tested and corresponding percent immobilization of Daphnia magna exposed to Biphenyl

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test condition :

The pH and dissolved oxygen ranged from 7.9 to 8.0 and 8.0 to 8.8 mg/L, respectively. The mean temperature was 22.2 deg C and the alkalinity and hardness ranged from 250 to 266 mg/L and 242 to 248 mg/L.

PARAMETER	CONC.	T I M E	
	(mg/L)	0-Hr	48-Hr
Temperature (°C)	Control	21.7	22.7
DO (mg/L)	Control	8.5	8.5
	0.65		8.8
	1.8		8.6
	5	8	8.4
pH	Control	7.9	8
	0.65		8
	1.8		8
	5	7.9	8
Alkalinity (mg/L)	Control	250	266
	0.65		260
	1.8		254
	5	256	250
Hardness (mg/L)	Control	246	242
	0.65		246
	1.8		242
	5	248	244
Conductivity	Control	800	700
	0.65		800
	1.8		725
	5	800	750

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Under these conditions, the 48-hour EC50 for Daphnia magna is 3.65 mg/L (confidence limits of 3.24 to 3.93) and the NOEC is 1.8 mg/

Reliability : (2) valid with restrictions

Although this study was not condusted under full GLPs, it was conducted by a scientifically defensible method following a standard laboratory guideline and is considered to have high reliability

13.12.2003 (14)

Remark : ECOSAR Program (v0.99f) Results:

==========

SMILES : c1ccccc1c2ccccc2

CHEM : Biphenyl

CAS Num:

ChemID1:

ChemID2:

ChemID3:

MOL FOR: C12 H10

MOL WT : 154.21

Log Kow: 4.01 (User entered)

Melt Pt: 70.00 deg C

Wat Sol: 7.48 mg/L (measured)

ECOSAR v0.99f Class(es) Found

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR	: Fish	14-day	LC50	3.676

(Baseline Toxicity)

Neutral Organics	: Fish	96-hr	LC50	1.475
Neutral Organics	: Fish	14-day	LC50	3.676
Neutral Organics	: Daphnid	48-hr	LC50	1.816
Neutral Organics	: Green Algae	96-hr	EC50	1.274
Neutral Organics	: Fish	30-day	ChV	0.263
Neutral Organics	: Daphnid	16-day	EC50	0.224
Neutral Organics	: Green Algae	96-hr	ChV	0.407
Neutral Organics	: Fish (SW)	96-hr	LC50	0.893
Neutral Organics	: Mysid Shrimp	96-hr	LC50	0.101
Neutral Organics	: Earthworm	14-day	LC50	228.056 *

Reliability : (2) valid with restrictions
09.02.2005

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : ca. 1.8 calculated
Limit test :
Analytical monitoring : no
Method : other: US EPA (1971). Algal assay procedure bottle test. National Eutrophication Research Program, Pacific Northwest Water Laboratory, Corvallis, OR
Year : 1971
GLP : no
Test substance : other TS: Therminol

Method : Culture and test procedures followed those of US EPA (1971). In brief, the study was designed to measure both decrease of in vivo chlorophyll a and a decrease in cell number over time. Algae were obtained from the US EPA Env. Res. Lab., Corvallis, OR, USA. At least 2 x 10E4 cells/mL were incubated at 24 deg C with 4000 lux illumination at 5 nominal test concentrations (1.0, 3.2, 5.6, 10 and 32 mg/L). Triplicate cultures were employed for each of the test concentrations and the controls. Test concentrations were prepared by adding appropriate weighed amounts of test material, dissolved in reagent grade dimethylformamide (DMF), to each flask. A solvent control was also maintained to which was added 0.05 ml of DMF, the maximum volume added to a test flask. Test material was not measured during the test. The test system was 125 ml flasks containing 50 mL test medium, the pH ranged between 7.1-7.6 throughout the study. The lower pH values were observed at the two highest nominal concentrations. Photoperiod was 24 hours light.

Measurement of in vivo chlorophyll a in cultures was performed by using a Turner Model III fluorometer. Cell counts were made using a hemacytometer and a Zeiss Standard 14 compound microscope.

Statistical analysis: Each test concentration was converted to a logarithm and the corresponding percentage decrease of in vivo chlorophyl a or cell numbers was converted to a probit (Finney, 1971). The 48-, 72-, and 96-hour EC50s and 95% confidence limits were then calculated by linear regression. To determine whether growth of the solvent control differed from that of the culture medium control, data were analyzed by "Student's" t-test (Steel and Torrie, 1960). Differences were considered significant at the 95% confidence level (p </- 0.05).

References:
Finney, D.J. (1971). Probit Analysis. Cambridge University Press. London. 333 pg.

Steel, R.G.D. and Torrie, J.H. (1960). Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York. 481 pg.

U.S. Environmental Protection Agency (1971). Algal Assay Procedure: Bottle test. National Eutrophication Research Program. Pacific Northwest Water Laboratory, Corvallis, OR 82 pg.

Remark : The observed response between two commercial products, one containing 73.5% diphenyl oxide and the other essentially pure diphenyl oxide, described in this document was essentially the same. Since the only difference between these two products was the presence of biphenyl, one can conclude that biphenyl does not contribute significantly to the toxicity of diphenyl oxide. In other words, the EC50 for biphenyl would be expected to be similar to that of the mixture of diphenyl oxide and biphenyl, approximately 1.8 mg/L.

Result : Based on a decrease of in vivo chlorophyll a, the estimated 24-hour EC50 was between 10 and 32 mg/L while the calculated 96-hour EC50 was 1.8 ppm with 95% confidence limits of 0.7-5.3 ppm (Table 1). The calculated 96-hour EC50, based on cell numbers, was 1.3 with 95% confidence limits of 0.6-7.2 ppm. After 96 hours of exposure, in vivo chlorophyll a was decreased 7% in cultures exposed to 1.0 mg/L to 100% in cultures exposed to 32 mg/L compared to control values (Table 2). A similar response was observed in the cell numbers at each dose level.

There was no significant difference between growth of the control and solvent control cultures after 96 hours of exposure, based on both in vivo chlorophyll a and cell numbers.

Test substance : Test material was identified as 26% biphenyl in the title with the remainder presumed to be diphenyl oxide. Solutia further identified the material as Therminol VP1 with a typical purity of 26.5% biphenyl and 73.5% diphenyl oxide (personal communication Elmer Rauckman).

Reliability : (2) valid with restrictions
08.02.2005 (15)

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 2.5 calculated
Limit test :
Analytical monitoring : no
Method : other: US EPA (1971). Algal assay procedure bottle test. National Eutrophication Research Program, Pacific Northwest Water Laboratory, Corvallis, OR

Year	:	1980
GLP	:	yes
Test substance	:	other TS: DPO unspecified but likely commercial grade with purity of >99%.
Method	:	Study procedures followed guidance found in The S. capricornutum Printz Algal Assay Test. Experimental Designs, Application and Data Interpretation. Corvallis, Environmental Research Laboratory, US EPA, 1978l. Study designed to measure both decrease of in vivo chlorophyll a and a decrease in cell number over time. Algae was obtained from the US EPA Env. Res. Lab., Corvallis, OR, USA. At least 2 x 10E4 cells/ml were incubated at 24 deg C wiht 4000 lux illumination at 5 nominal test concentrations (0.6, 1.2, 2.5, 5 and 10 mg/L). Both an untreated control and a solvent (triethylene glycol) control groupwere also included in the test. All test concentrations were conducted in triplicate. Test material was not measured during the test. The test system was 125 ml flasks containing 50 ml test medium, the pH ranged between 7.2-7.6 throughout the study. Photoperiod was 24 hours light. Chlorophyll measurements wree taken using a fluorometer; cell counts were made using a hemacytometer and compound microscope. Study procedures followed guidance found in The S. capricornutum Printz Algal Assay Test. Experimental Designs, Application and Data Interpretation. Corvallis, Environmental Research Laboratory, US EPA, 1978l. Study designed to measure both decrease of in vivo chlorophyll a and a decrease in cell number over time. Algae were obtained from the US EPA Env. Res. Lab., Corvallis, OR, USA. At least 2 x 10E4 cells/mL were incubated at 24 deg C with 4000 lux illumination at 5 nominal test concentrations (0.6, 1.2, 2.5, 5 and 10 mg/L). Both an untreated control and a solvent (triethylene glycol) control group were also included in the test. All test concentrations were conducted in triplicate. Test material was not measured during the test. The test system was 125 ml flasks containing 50 mL test medium, the pH ranged between 7.2-7.6 throughout the study. Photoperiod was 24 hours light. Chlorophyll measurements were taken using a fluorometer; cell counts were made using a hemacytometer and compound microscope. Data were treated statistically by using the probit method of Finney (1971) followed by linear regression analysis. A probability factor of 5% was used. References: Finney, D.J. (1971). Probit Analysis. Cambridge University Press. London. 333 pg.
Remark	:	Although this study appears to have been conducted using an experimental design published in 1978, the method appears to be nearly identical, if not identical, to the earlier method used for Therminol VP-1. Thus the results from the two studies should be equally valid. Information copied from IUCLID dossier for diphenyl oxide (CAS # 101-84-8) http://www.epa.gov/chemrtk/diphoxid/c14164rr.pdf
Result	:	Based on the decrease in chlorophyll the following EC50 values (95% CI) were calculated: 96-hr = 2.5 (1.2-5.4) mg/L; at 72-hr and 48-hr the EC50 value was between 2.5 and 5.0 mg/L and at 24-hr the EC50 value was >10 mg/L. Based on the number of decreased cells, the 96-hr LC50 (95% CI) = 2.5 (1.2-5.3) mg/L.
Reliability	:	(2) valid with restrictions
07.02.2005		(16)
Species	:	Chlorella vulgaris (Algae)
Endpoint	:	growth rate
Exposure period	:	3 hour(s)
Unit	:	mg/l

5. Toxicity

Id 92-52-4
Date 02.05.2005

EC50 : = 3.9 measured/nominal
Method :
Year :
GLP : no data
Test substance :

Method :

Cultures of the green alga Chlorella vulgaris (#260 Indiana collection) were grown under axenic conditions using Bold's Basal medium (BBM) in cotton-plugged 125 m1 Erlenmeyer flasks. The media was at a pH of 6.5 and a 12-hour light-dark cycle was used with a light intensity of 400 foot candles, and a temperature of 19°C.

Saturated solutions were prepared by stirring the solid biphenyl in sterile BBM for 24 hours. The saturated solution was then decanted or filtered, and dilutions made with BBM to provide 0, 20, 50 and 100 percent of the original saturation level of biphenyl.

Radiolabeled carbon dioxide (carbon 14) uptake was used as a measure of photosynthesis. Radiolabel was added to provide an activity of 0.5 p Ci/100 ml.

Three-to-four day exponential phase alga cells were used for experiments, at a cell concentration of 20 x 104 cells/ml. Labeled carbon dioxide was added at time zero, the flasks were sealed with a glass stopper and were incubated under the described conditions for three hours. A dark set of controls was also run. After filtering, the cells were washed with 0.85 percent saline to remove surface sodium bicarbonate and radioactivity was determined on dried Millipore filters using an end window radiation detector (Nuclear Chicago)

Remark :

Although this determination of inhibition was of a short duration, sensitive and precise measures of growth were employed that are considered to provide an accurate estimate of the IC50. In addition, the high volatility of biphenyl from open aqueous systems (Henry's Law constant 2.5 x 10-4 atm-m3/mol) indicates that loss of test material over a period of hours will be significant. (A volatilization half-life of 4.3 hours was estimated for biphenyl in a stream 1 m deep, flowing 1 m/second, with an air current of 3 meters/second*). Thus, studies in open systems of duration longer than a few hours are not anticipated to be robust tests of inhibition due to the constant and rapid reduction of test material concentration.

* USEPA. Health and Environmental Effects Profile for 1,1'-biphenyl. Environmental Criteria and Assessment Office, Cincinnati, OH, 35 pp 1984

Result :

Percent inhibition of radiocarbon uptake at each dilution 0, 20, 50 or 100% of saturation was plotted and the concentration that caused a 50% inhibition (3.9 mg/L) was determined. Thirty-eight different hydrocarbons were tested using essentially the same procedure and the IC50 values were plotted versus both water solubility and Kow (log-log plot). A regression line was drawn through the data and the fit was found to be good with a correlation coefficient between 0.80 and 0.93. Data from biphenyl fell precisely on the regression line indicating high precision for this IC50 estimate

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance :

Biphenyl, CASNO 92-52-

Conclusion :

The 3-hour IC50 for growth of the green alga Chlorella vulgaris was determined to be 3.9 mg/L under these conditions

Reliability : (2) valid with restrictions

Well-documented published study using a sensitive and precise means of measuring algae growth inhibition

07.02.2005 (17)

Remark : ECOSAR Program (v0.99f) Results:

==========

SMILES : c1ccccc1c2ccccc2

CHEM : Biphenyl

CAS Num:

ChemID1:

ChemID2:

ChemID3:

MOL FOR: C12 H10

MOL WT : 154.21

Log Kow: 4.01 (User entered)

Melt Pt: 70.00 deg C

Wat Sol: 7.48 mg/L (measured)

ECOSAR v0.99f Class(es) Found

Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR	: Fish	14-day	LC50	3.676
(Baseline Toxicity)				
Neutral Organics	: Fish	96-hr	LC50	1.475
Neutral Organics	: Fish	14-day	LC50	3.676
Neutral Organics	: Daphnid	48-hr	LC50	1.816
Neutral Organics	: Green Algae	96-hr	EC50	1.274

Neutral Organics	: Fish	30-day	ChV	0.263
Neutral Organics	: Daphnid	16-day	EC50	0.224
Neutral Organics	: Green Algae	96-hr	ChV	0.407
Neutral Organics	: Fish (SW)	96-hr	LC50	0.893
Neutral Organics	: Mysid Shrimp	96-hr	LC50	0.101
Neutral Organics	: Earthworm	14-day	LC50	228.056 *

Reliability : (2) valid with restrictions
09.02.2005

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

Species : Salmo gairdneri (Fish, estuary, fresh water)
Endpoint : other: hatching, survival, swiw up, growth
Exposure period : 87 day(s)
Unit : mg/l
NOEC : = .229 measured/nominal
LOEC : = .332 measured/nominal
MATC : = .275 calculated
Analytical monitoring : yes
Method :
Year :
GLP : yes
Test substance :

Method :

Animals: Rainbow trout (Salmo gairdneri Richardson) used in acute testing were obtained as eyed embryos from 'Mt. Lassen Trout Farms, Red Bluff California on March 11, 1987. Upon arrival they were placed in a trout hatcher and incubated at 12 ± 2°C until hatched. Juveniles were held in 110 L stainless steel aquaria at a water temperature of 12 ± 2°C, and were provided a 16-h light/8-h dark photocycle. A synthetic diet was provided ad libitum. Juvenile trout ca. 160 days post-hatch were acclimated to test temperature at least 72-h prior to testing.

Six test concentrations of biphenyl, an acetone control, and a water control; were used in this study each concentration was in quadruplet. Embryos less than 96-h post fertilization were removed from the trout hatcher. The test was started by impartially distributing 50 embryos to each embryo cup. The distribution procedure was as follows: 10 embryos (20% of total) were impartially selected and transferred with a large bore pipette to successive incubation cups; this process was repeated until 50 embryos were in each cup. The embryo cups were randomly placed in test aquaria. During the embryo exposure phase, the embryos were shielded from direct light by black polyethylene curtains. Care was taken not to jar, move or otherwise shock the embryos during incubation, especially during the sensitive pre-eyed stage, from about 7 days post fertilization to the eyed stage. Once the

eyed stage was discernable, embryos were thinned to 30 embryos per replicate (120 per treatment). Thereafter, the embryos were observed daily; dead embryos were counted and removed at each observation. Upon completion of hatching, the total number of alevins in each replicate, including those dead or deformed, was counted. Dead or deformed alevins were subtracted from the total to determine the number of normal alevins at hatch. Also, the percent of embryos that hatched and the day-to-mean hatch in each replicate was recorded.

Alevins were observed at least 3 times weekly with mortality and developmental abnormalties recorded. At swim-up, alevins were released into the test vessel (time to 50% swim-up was recorded for each test concentration). The test continued for 60 days post day-to-mean hatch of controls. Post swim-up alevins were observed at least three times a week with mortality and behavioral or other sublethal effects recorded at each observation. At termination all surviving fish were sacrificed for weight and length measurements.

Water: The water supply is pumped from the Upper Saginaw Bay of Lake Huron. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. As it enters he laboratory, the water is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the test; pH 7.4 to 7.8 hardness (mg/L as calcium carbonate) 72 to 80 alkalinity (mg/L as calcium carbonate) 45 to 52 and conductivity 150 to 190 (umhos/cm).

Dilutor: An intermittent-flow proportional diluter system was used. This system was designed to deliver six test concentrations, a carrier and water control. The diluter was calibrated so that the concentration of the test material in each treatment below the high concentration was approximately 65 percent of that in the next higher treatment level. The carrier control received acetone at a concentration equaling the highest concentration in any treatment group, (no more than 0.1 ml/L). The diluter operates as follows: a precision dosing system delivers the test material from a stock bottle to a mixing chamber where it is mixed with dilution water and then distributed to "toxicant cells". When the diluter cycles, the test material from each toxicant cell blends with water from its respective dilution water cell and then flows into mixing/splitting chambers. Silicone delivery tubes from these chambers provide approximately 500 mL to the test aquaria, which are positioned on one tier, side by side, in a temperature-controlled water trough. The diluter was calibrated prior to the beginning of the tests and was found to be operating normally. The diluter was set to provide at least 15 volumes turnovers in the test aquaria each twenty-four hours.

The test vessels were constructed of double-strength glass glued with clear silicone adhesive, and measure approximately 30 x 15 x 14 cm deep. Each is provided with a nylon screen covered drain that maintains a water volume of 3.7 liters. In the embryo-larval test, the embryos were incubated in circular (124 mm in diameter by 51 mm high) cups with 360 um nylon screen bottoms that were supported in the test vessels by glass beads. The flow from the delivery tube was directed into the incubation cup to produce a flow of water around the embryos during the incubation period.

The analysis schedule was as follows: Embryo-larval test: on days 0, 1, 3, 6, 9, 10, 13, 15, 22, 24, 29, 30, 34, 36, 42, 44, 45, 48, 51, 52, 55, 58, 62, 63, 64, 69, 71, 76, 77, 79, 83, and 87. Also, during the embryo-larval test, the biphenyl concentration was measured in each test vessel on days 0, 15, 30, 55, and 83; whereas a composite of all four replicates was analyzed at each concentration on the remaining days.

Statistics: For analysis of the embryo-larval data, the percent of embryos hatched, normal larvae at hatch, survival and unweighted replicate means of length and weight data were evaluated by one-way analysis of variance procedure. The Dunnett's two-tailed t-test was used to compare treatment means to water and acetone control means independently, at a = 0.05. Day-to-mean hatch and day-to-mean swim-up were assessed by linear regression analysis ($p < 0.05$)

Result :

Analytically determined concentrations were:
(Representative values and means)

Day 0 (mg/L)	Day 30 (mg/L)	Day 87 (mg/L)	Mean ± S. D. (n = 28 to 31)
0.418	0.544	0.508	0.554 ±0.091
0.288	0.351	0.312	0.332 ±0.039
0.220	0.249	0.218	0.229 ±0.028
0.136	0.147	0.137	0.143 ±0.015
0.096	0.103	0.097	0.099 ±0.014
0.063	0.065	0.060	0.063 ±0.008

Analyzed biphenyl concentrations ranged between 85 and 94 percent of nominal (see table for representative values and means). Testing was terminated 61 days following day-to-mean hatch of the water controls. Embryos were culled to 30 embryos per replicate on day-20 post fertilization. Evaluation of the number of viable embryos showed that fertilization success was 84%. The day-to-mean hatch was day 26 for the water controls; day 27 at 0.564, 0.332, 0.229 mg/L and the acetone controls; and day 28 at 0.143, 0.099 and 0.063 mg/L. The day-to-mean swim-up at the high concentration (0.564 mg/L) was day 43 and was delayed five days (statistically significant, p=0.05) when compared to the controls and other treatment groups. Comparing either the water controls or acetone controls to the treatment groups statistical analysis showed no differences in the number of embryos that hatched, number of deformed larvae and larval survival; however, there was substantial, but not statistically significant, mortality at the high concentration.

Terata were unrelated to exposure concentration and included spinal and cranial deformities, poorly developed yolk sac, and small, poorly pigmented individuals. There was no difference in growth, as indexed by weight or length, between the acetone and water controls. Nevertheless, both sets of control data were used independently for comparative purposes in statistical analyses. Weight and length varied among the treatment groups and the controls. Weight was significantly reduced ($p = 0.05$) at 0.560 mg/L, and at the two lowest concentrations 0.099 and 0.063 mg/L. Length was significantly reduced at 0.564, 0.332 and 0.063 mg/L.

Hatching and early parameters

Conc. (mg/L)	Embryos Hatched	Hatch (day)	Deformed Larvae(%)	Swim-up (day)
0.564	95.8±6.3	27	3.4 ±2.7	48*
0.332	97.5±3.2	27	2.7 ±1.7	43
0.229	97.5±3.2	27	2.6 ±3.4	43
0.143	95.0±4.3	28	0.83±1.7	43
0.099	99.2±1.7	28	0.83±1.7	43
0.063	98.3±1.7	28	0.83±1.7	43
Cont(A)	98.3±3.3	27	2.6 ±1.7	43

Cont(W)	100.0±0.0	26	0.83±1.7	43

Growth and Survival

Conc. (mg/L)	Survival %	Length (mm)	Wet Weight (mg)
0.564	64.2±21.8	29.2±0.84*	366±0.04*
0.332	75.0±21.9	32.6±1.5*	583±0.07
0.229	81.7±23.8	34.8±0.55	662±0.02
0.143	72.5±15.2	33.9±1.1	604±0.06
0.099	84.2±11.0	33.0±0.62	545±0.03*
0.063	89.2±1.7	32.7±0.61*	514±0.03*
Cont(A)	79.2±10.7	34.7±0.53	647±0.04
Cont(W)	83.3±26.8	34.7±0.53	652±0.03

A = Acetone (solvent) control, W= water control

In view of the lack of well defined concentration-responses for parameters evaluated in this test (see tables), the implications of the embryo-larval data must be based on a toxicological as well as a statistical assessment of the data. Considering all the available data, the most scientifically sound assessment of the data and the one that most accurately reflects the potential chronic effects of biphenyl to fishes is the following.

The NOEC is 0.229 mg/L and the lowest effect concentration is 0.332. mg/L. The MATC based on the geometric mean of these two values is 0.275 mg/L. This assessment is supported by the integration of statistical and toxicological assessments of the data. This analysis recognizes statistically significant reduction on growth at 0.564 and 0.332 mg/L while at the same time rejects the statistically significant reduction in growth at 0.099 and 0.063 mg/L as spurious. This interpretation is based on a clear concentration-response relationship in growth and survival between 0.229 and 0.564 mg/L

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test condition :

----------CONDITIONS----------

Temperature	12 ± 1°C
Photoperiod	16 hrs light/8 dark
Aeration	None
Type of Test	Early Life Stage
Test Vessel Size	Approximately 30x15x14 cm deep
Test Volume	3.7 L
No. of Treatment groups	6
No. of Replicates/Treatment	4
Organisms/Replicate	50 culled to 30
Dissolved Oxygen	75% or greate

Test substance :

Biphenyl CASNO 92-52-4, purity > 99.1

Conclusion :

Under the conditions of this early-life-stage study, the NOEC is considered to be 0.229 mg/L biphenyl and the MATC is assigned as 0.275 mg/L

Reliability : (1) valid without restriction

High quality guideline-like study under GLP with analytical suppor

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	other: reproduction and growth
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	= .17 measured/nominal
LOEC	:	= .32 measured/nominal
MATC	:	= .23 calculated
Analytical monitoring	:	yes
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

Animals: Daphnia magna Straus, 1820, was used as the test organism in this study. The daphnids were cultured in the laboratory from parthenogenetic females. On the day before testing began, reproductively mature females were isolated. Young produced by these adults were collected and used for testing within 24 hrs.

Vessels for this study were 600 ml glass beakers with a notch cut in the lip to facilitate water drainage from the vessel. Each test beaker contained five glass tubes (2.5 x 12.5 cm with 363 um mesh bottoms) that were set on the bottom of the beaker. The test unit design was such that the test and control solutions entered the tops of the tubes and eventually passed through the mesh bottom and exited through the notch cut in the beaker. The beakers were maintained in a temperature controlled water trough.

The chronic test began with the placement of one neonate daphnid (<24 hrs old) in each of the five tubes contained within a beaker. The tubes were given unique labels and each daphnid remained in a particularly labeled tube for the entire study. There were four replicate beakers for each concentration (5 concentrations), and for the water and carrier controls (acetone 0.1 ml/L) resulting in 20 daphnids being exposed to each concentration. The duration of the chronic test was 21 days.

Each test beaker contained approximately 400 ml of the appropriate amounts of test material, food and dilution water. The daphnids were fed a diet of S. capricornutum at the rate of ca. 2.50 mg dry wt/L of dilution water twice daily. Each Monday, Wednesday and Friday the young produced by each adult were counted and discarded and adult survival was recorded. Dissolved oxygen, pH and temperature were measured daily from a high, middle, low and control replicate.

Analysis: The concentration of biphenyl in the test and control solutions was analyzed using reverse-phase high performance liquid chromatography. Biphenyl was detected using a U.V. detector set at 254 nm. The linearity of the detector was assessed by making biphenyl standards in dilution water. The biphenyl levels in the samples were quantified using an external standard technique, in which a response factor for a particular analysis day was calculated and applied to the samples to determine the biphenyl concentrations. The test material was used to make all analytical standards and fresh standards in dilution water were prepared each analysis day. Analyses occurred at a minimum on days -1, 0, 7, 14 and 21. On each sampling day, samples were taken from two replicates from each test concentration and the controls. Additional samples were taken on days 2, 5, 13 and 19.

Water: The water supply is pumped from the Upper Saginaw Bay of Lake

Huron. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. As it enters he laboratory, the water is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the test; pH 7.4 to 7.7, hardness (mg/L as calcium carbonate) 73 to 78, alkalinity (mg/L as calcium carbonate) 49 to 52 and conductivity (umhos/cm) 160 to 170.

Dilutor: Testing was conducted with an intermittent-flow proportional diluter equipped with a Micromedic automatic pipette, which was triggered to inject the appropriate amount of test material into the toxicant mixing chamber at the beginning of each cycle. The toxicant mixing chamber was equipped with a recirculating pump that provided mixing for at least three minutes before the solution was delivered to the testing chambers. The diluter had a dilution factor of about 0.50. At each cycle 500 ml test solution or control water was delivered to each flow-splitting dilution chamber. These chambers, which were randomly positioned on the diluter, diverted ca. 125 ml to each of four replicate test chambers at each test concentration and the control during the acute test. During the chronic study, the 125 ml delivered from the Splitter cells to each replicate was split five ways into each of the five tubes contained within a replicate beaker. The diluter was set to cycle every 30 minutes resulting in a minimum of 15 volume replacements in each beaker per day.

Statistics: Data derived from the chronic portion of this study were analyzed using analysis of variance followed by Dunnett's t-test (alpha = 0.05). Mean comparisons between test and control concentrations were performed on the following parameters at the end of the test: percent survival, mean total young/daphnid, growth (as weight) and mean brood size. The purpose of these comparisons was to estimate the maximum acceptable toxicant concentratio

Result :

The mean analyzed concentrations ranged from 90.3 to 132% of the corresponding nominal values. Over the course of the 21-day study the variation of analyzed solutions within a concentration was very small.

During the 21-day chronic study there was no mortality observed in either the acetone or water controls. Throughout the test the dissolved oxygen measurements were >90% saturation (range 8.4 to 8.8 mg/L). The pH and temperature ranged from 7.4 to 7.8 (pH within a concentration remained within 0.3 units) and 20.0 to 20.6°C, respectively.

Conc	Surv Size	Brood per adult	Mean young Per adult	Mean dry wt
0.56	0*	0*	0*	0*
0.33	20*	10.1±1.8*	34.0±10*	135±92
0.17	100	18.6±0.5	67.2±3.1	274±76
0.07	100	18.2±0.4	64.7±2.2	285±88
0.03	100	18.2±0.2	67.5±2.5	280±48
Water	100	18.9±0.4	63.1±2.6	217±52
Acetone	100	18.6±0.3	67.0±2.3	238±34

surv = survival * p < 0.05

The data used to estimate the MATC are presented above. There were no statistically significant differences between the controls based on survival, mean brood size, mean number of young per adult and mean dry weight. For the purpose of estimating the MATC, the data derived from the water controls were compared to those of the various treatment groups. Interpretation of the chronic data indicates that the MATC lies between 0.17 and 0.32 mg/L and is 0.23 mg/L expressed as the geometric mean of these two concentrations. The no observed effect concentration was 0.17 mg/L. The estimation of the MATC was based on data associated with survival and reproduction (mean brood size and mean total young per

adult). These endpoints both significantly differed from the controls at the 0.32 mg/L concentration. No other toxic effects were observed.

During the study the first young born in the controls were observed on days 8 and 9. Additionally, the average number of young per adult in the controls exceeded 40 and there were no ephippia produced by any of the test organisms.

Dividing the chronic value generated during this study (0.23 mg/L) into the acute value (0.36 mg/L) results in an acute/chronic ratio of 1.6. Based on the acute/chronic ratio calculated for biphenyl it would be unlikely to observe chronic invertebrate effects much below levels that are acutely toxic

Source : Toxicology and Regulatory Affairs Freeburg, IL

Test condition :

TEST CONDITIONS

Temperature	20 ± 1°C
Photoperiod	16 hrs light/8 dark
Daphnid source	laboratory reared
Diet	Selenastrum capricornutum
Test Vessel	600 ml beaker
Observations	D.O., pH, temperature, morality, offspring MWF
Effect Criteria	reproduction, mortality, growth (as weight)
Length of Test	21 day

Test substance :

Biphenyl, purity > 99.2% CASNO 92-52-4

Conclusion :

In this 21-day reproduction study, the MATC is 0.23 mg/L and the NOEC is 0.17 mg/L. The MATC is based on data associated with survival and reproduction (mean brood size and mean total young per adult)

Reliability : (1) valid without restriction

High quality guideline-like study under glp with analytical support.

13.12.2003 (19)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Metabolism
Species : rat
Number of animals
Males : 5
Females :
Doses
Males : 1.6% biphenyl + 5% KHCO3
Females :
Vehicle :
Route of administration : oral feed
Exposure time : 7 day(s)
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st.:
2nd.:
3rd.:
Toxic behaviour :
Deg. product :

Method : Chemicals used were from the same sources as outlined by Ohnishi et al., 2001.

Groups of 5 male F344/DuCrj rats (Charles River Japan, Inc. (Atsugi, Japan) were fed 1.6% biphenyl and 5.0% KHCO3 for 7 days. Urine samples were collected from 5 rats fed the biphenyl-containing diet between days 6 and 7. Samples were pooled as one sample. Precipitates were separated from urine by centrifugation and then dissolved in acetonitrile. Th acetonitrile solution was centrifuged and the supernatant evaporated under vacuum at 40C to obtain the urine crystals. The remaining urine diluted with acetonitrile or the urine crystals dissolved in acetonitrile were used for LC-MS/MS analyses of the biphenyl sulfate conjugates.

LC-MS/MS chromatography:
Biphenyl metabolites in the urine, urine crystals and standard samples were separated by LC-MS/MS. A Tosoh TSK-Gel ODS-80TS column (150 x 2.0 mm id)at a flow rate of 0.25 ml/min with the column at ambient temperature was used.

Result : As shown in Table 1, 3-hydroxy-biphenyl-O-sulfate (3-HBPOS), 4-hydroxy-biphenyl-O-sulfate (4-HBPOS) and 3,4-dihydroxybiphenyl-3-O-sulfate (3,4-DHBP-3-OS) accounted for more than 70% of the total in male urine. Nearly 90% of the urine crystals was composed of 4-HBPOS.

The higher concentration of 4-HBPOS in the urine crystals is believed to be due to the lower solubility of this material as compared to the other sulfate conjugates.

Attached document :

Table 1. Content of Biphenyl Sulfate Conjugates in Rat Urine Including Urine Crystals

Biphenyl sulfate conjugates	Urine (%)	Urine crystals (%)
2-HBPOS (Peak 1)	3.32[a)]	0.06
3-HBPOS (Peak 2)	23.37	1.06
4-HBPOS (Peak 3)	11.94	89.45
4,4′-DHBPOS (Peak 4)	7.17	3.11
2,5-DHBPOS (Peak 5)	5.62	0.02
3,4-DHBP-3-OS (Peak 6)	40.88	3.90
3,4-DHBP-4-OS (Peak 7)	2.27	2.28
2,3-DHBPOS (Peak 8)	5.43	0.12

a) The component fraction (%) for each of the sulfate conjugates was estimated from the ratio of the LS-MS/MS peak area of the sulfate to the total area.

Test substance : Biphenyl was obtained from Wako Pure Chemical Ind (Osaka, Japan) Although no purity information was provided in the report. Test material for other studies from this same company was >98% pure (Umeda et al., 2002).

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment.

01.03.2005 (20)

In Vitro/in vivo : In vitro
Type : Absorption
Species : human
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle :
Route of administration : dermal
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st.:
2nd.:

3rd:

Toxic behaviour :
Deg. product :
Method : other: OECD 428
Year : 2004
GLP : yes
Test substance :

Method : The permeability coefficient (Kp) and the short-term absorption rates at 10 and 60 minutes were determined for biphenyl using human abdominal skin from cadavers mounted in an in vitro static diffusion cell model. Following system equilibration, skin integrity was confirmed by electrical impedence (EI). The saline in the donor and receptor chambers was removed, discarded and the donor chamber filled with 0.9% saline fortified with 6% polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether).

For the permeability coefficient experiment, an infinite dose of biphenyl in isopropyl myristate vehicle (100 ul/cm2) was applied to the epidermal surface, via the donor chamber to 6 skin replicates representing 3 human subjects, and the donor chamber opening was occluded with Parafilm. Serial receptor fluid samples were taken at 1, 2, 4, 8, 12, 24, 36 and 48 hours post-application and analyzed for radioactivity by liquid scintillation counting. At the end of the 48-hour exposure, excess biphenyl was removed by washing with a 2% soap solution followed by rinsing with water. The receptor fluid was removed and discarded, and the receptor and donor chambers were filled with 0.9% saline and an end of experiment integrity assessment was determined using electrical impedence (EI).

For the short-term exposure experiments, a finite dose of biphenyl in isopropyl myristate vehicle (20 ul/cm2) was applied to the epidermal surface, via the donor chamber, to 12 skin replicates representing 3 human subjects, and the donor chamber opening was occluded with Parafilm. At the end of the required exposure interval (10 minutes and 60 minutes), 6 replicates each were terminated. At termination, the skin surface was washed with a 2% soap solution, rinsed with water, and the receptor fluid was removed and retained for analysis. The receptor and donor chambers were filled with 0.9% saline and end of experiment integrity assessment was determined using EI. The saline in both chambers was removed and discarded and the skin membrane removed and placed into a glass vial containing methanol. The receptor fluid and the skin were analyzed by liquid scintillation counting.

Test substance : Biphenyl non-labeled material was 99.8% pure and labeled material was >99% pure.

Result : Based on the slope at steady state (3.93 ug equiv/cm2/hour), and the concentration of the applied dose of biphenyl (100,263 ug/cm3), the permeability coefficient was calculated to be 3.92 x 10(-5) cm/hr.

Following a 10-minute exposure to a finite application of biphenyl, a total of 0.16 ug equivalents in the skin. Based on the amount of biphenyl in the receptor fluid and skin, an exposure area of 0.64 cm2 and an exposure time of 10 minutes (0.17 hours), the short-term exposure rate was calculated to be 165.3 ug equivalent/cm2/hr.

Following a 60-minute exposure to a finite application of biphenyl, a total of 1.32 ug equivalents of biphenyl was detected in the receptor fluid and 27.3 ug equivalents in the skin. Based on the amount of biphenyl in the receptor fluid and skin, an exposure area of 0.64 cm2 and an exposure time of one hour, the short term exposure rate was calculated to be 28.6 ug equivalent/cm2/hr.

Reliability : (1) GLP guideline study

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 2400 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals :
Vehicle : other: corn oil
Doses : 2000, 2510, 3160 or 3980 mg/kg bw

Method : The undiluted test substance was administered as a 20% solution in corn oil to Sprague-Dawley rats at four dose levels using two or three animals of each sex per group. Treated animals were observed for 14 days, survivors were sacrificed and subjected to an examination of the viscera. Body weights were only reported at the time of dosing (presumably used to determine the volume of test material to administer). Rats of each sex were used and all were in an initial weight range of 210 to 235 grams. Dose levels and animals per group are given in the results.

No additional information provided.

Remark : Most likely Monsanto production material from 1976 was tested.

Result : Dose levels, grouping and mortality were as follows:

	MORTALITY	
Dose(mg/kg)	Males	Females
2,000	1/3	0/2
2,510	1/2	2/3
3,160	1/3	2/2
3,980	2/2	3/3

Deaths occurred one to five days after dosing with most occurring within two days. Clinical signs reported were loss of appetite and activity for two to six days following administration and for moribund animals, increasing weakness, ocular discharge, collapse and death.

Necropsy revealed hemorrhagic areas of the lungs, slight discoloration of the liver and gastrointestinal inflammation.

Test substance : Biphenyl, CAS# 92-52-4.

Conclusion : The oral LD50 is 2,400 mg/kg with a 95% confidence limit of 2,180 to 2,640 mg/kg in Sprague-Dawley rats of combined sex.

Reliability : (2) valid with restrictions
Good documentation for an older study. Considered reliable but downgraded to 2 due to lack of individual animal data

Flag : Critical study for SIDS endpoint

08.02.2005 (22)

Type : LD50
Value : = 3280 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : no data
Number of animals : 60
Vehicle : other: olive oil
Doses :

Method : Sprague-Dawley rats were administered purified Biphenyl as a 25% solution in olive oil by gavage. Group size and dose levels were not specified except that it was reported that 60 rats were utilized to determine the oral LD50 of Biphenyl in the rat. Six other materials were also reported on in the publication. After dosing rats were observed for adverse clinical

signs. It is not stated how long the observation period was after dosing. It is stated that one rat exposed orally to Biphenyl died 2 days after dosing and it is noted that some animals in the larger study survived up to 18 days after dosing. It is, therefore reasonable that the post-dosing observation was 18-days. Evidence that necropsy examinations were performed comes from statements concerning the local effects of Biphenyl on the GI tract

Result : The purified-Biphenyl oral LD50 for rats is listed as 3.28 g/kg in a table. It is also listed that 60 rats were used to make this determination and the survival time varied from 18 hours to 2-days. Dose levels and mortalities are not provided. Clinical signs of toxicity are noted generally for all compounds as "inducing a state of intoxication characterized by an increased respiratory rate. Lacrimation, loss of appetite, loss of body weight, muscular weakness, unsteadiness and respiratory difficulties, and terminated by death in coma." Other compounds that were studied are o- and p-aminodiphenyl, o- and p-nitrodiphenyl and dihydroxyoctachlorodiphenyl.

Necropsy results from animals dying on test are generically described as causing little or no local injury, except for slight irritative effects in the stomach, duodenum and upper jejunum of animals that died within a few hours after dosing.

The report mentions that effects on the kidneys and liver were observed but the report does not indicate if these were from Biphenyl or other Biphenyl derivatives that were dosed. Likewise slight to severe toxic degenerative changes in the myocardium were reported but it is not clear if these were associated with Biphenyl of the other compounds.

Test substance : Biphenyl, purified. CASNO 92-52-4

Conclusion : The oral LD50 of Biphenyl in the Sprague-Dawley rat is 3280 mg/kg body weight.

Reliability : (2) valid with restrictions
Published reports are assigned a reliability of 2. Despite differences from the current guideline and the lack of details that would be reported in a modern investigation, the study appers to have been well conducted. This study also uses more animals than other determinations of LD50 for this material and may be the most accurate determination of the LD50.

01.03.2005 (23)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value :
Species : rat
Strain : other: CFE
Sex : female
Number of animals : 6
Vehicle : other: air
Doses : saturation
Exposure time : 8 hour(s)

Method : A group of 6 female CFE albino rats weighing 126 to 131 grams were exposed for 8 hours to vapors, mists and decomposition products of Biphenyl produced by passing air at a rate of 2.5 L/min through a fritted glass disk immersed one inch into 50 ml heated Biphenyl in a bubbler, which was in turn submerged in a silicone bath at 176 deg C. The inhalation chamber was 9-L in volume. Liquefied Biphenyl in the bubbler never exceeded 166 deg C in temperature and the air temperature in the chamber averaged about 27 deg C. Animals were observed for 14 days after exposure.

Result	:	No animals died during the exposure or subsequent observation period. All animals gained weight (50 to 65 grams) during the observation period and no gross pathology was found at sacrifice.
Source	:	Toxicology and Regulatory Affairs Freeburg, IL
Test substance	:	Biphenyl, purified, ca. 99%. CASNO 92-52-4
Conclusion	:	Inhalation of saturated vapors of purified (ca 99%) biphenyl for 8-hours did not produce any mortality in female rats.
Reliability	:	(2) valid with restrictions Good documentation for an older study. Considered reliable but downgraded to 2 due to lack of details, including measurement of concentration and details of clinical observations.

01.03.2005 (24)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 5010 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: Corn oil
Doses	:	
Method	:	The test substance was administered as a 40% solution/suspension in corn oil to the closely-clipped skin of New Zealand white male or female rabbits weighing 2.0 to 2.2 kg at dosing. The exposure period is listed as 24 hours and it was typical at that laboratory to cover the exposed skin with plastic that was held in place for 24 hours but the exact conditions are not specified. Animals were observed for 14 days after treatment, sacrificed and necropsied. Increasing incremental doses were used to minimize animal usage.
Result	:	Dose levels and grouping were as follows:

Dose (mg/kg)	Animals	Result
5,010	1 F	no deaths
7,940	1M&1F	male died

Clinical signs reported were loss of appetite and activity for two to three days following administration in survivors and increasing weakness, collapse and death for the decedent. Necropsy of the animal that died indicated lung and liver hyperemia, slightly enlarged gall bladder and gastrointestinal inflammation

Source	:	Toxicology and Regulatory Affairs Freeburg, IL
Test substance	:	Biphenyl, CASNO 92-52-4
Conclusion	:	The Dermal LD50 is greater than 5010 mg/kg in New-Zealand rabbits.
Reliability	:	(2) valid with restrictions Good documentation for an older study. This study is considered an adequate test of approximate dermal toxicity. Procedure is similar to current OECD-423 Acute Toxic Class Method. Only study available that used appropriate vehicle.
Flag	:	Critical study for SIDS endpoint

01.03.2005 (22)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	Chronic
Species	:	rat
Sex	:	
Strain	:	
Route of admin.	:	oral feed
Exposure period	:	750 days
Frequency of treatm.	:	Constant
Post exposure period	:	
Doses	:	10, 50, 100, 500, 1,000, 5,000 or 10,000 ppm
Control group	:	yes, concurrent vehicle
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method :

Groups of 15 weanling rats of each sex were placed on diets containing seven levels of biphenyl for a period of 750 days. Animals were housed 5 to a cage and had free access to food and water at all times. During the period of growth, rats were weighed once a week and food consumption was determined weekly. Following the period of active growth, the rats were weighed at 50-day intervals for the duration of the study. Animals were examined at the time of weighing for gross evidence of tumors. At sacrifice, animals were necropsied, weights of liver, kidneys, heart, and testes were determined. Hematoxylin-eosin stained sections of heart, lung, liver, kidney, adrenal, spleen, pancreas, stomach, intestine, bladder, thyroid, brain, pituitary, and gonads were prepared and bone marrow smears of representative animals were prepared.

Dosed feed levels for the study were (in ppm) 0, 10, 50, 100, 500, 1000, 5000 or 10000 ppm (0.001 to 1%).

Studies on possible reproductive effects and survival of young were also conducted as follows. Ten weanling female and five males rats were placed on control diet for 60 days, and subsequently mated, one male to two females. An identical experiment included Biphenyl at a dietary level of 0.1%. Nine female and 3 male rats were fed a dietary level of 0.5% Biphenyl in a subsequent study. All rats continued exposure until the pups of all litters were weaned.

In a second series of experiments, 90-day old rats were exposed for 11 days before mating and continuously until weaning of pups. Using this dosing schedule, 8 female and 4 male rats were placed on the control diet, 8 females and 4 males received 0.1%, and 9 females and 3 males received 0.5% dietary levels of Biphenyl.

No additional information provided.

Result : Survival of animals was only reduced at the two highest concentrations. Details are shown in the table below.

Number of surviving animals/group-time:

		Days on Test											
	ppm	0	50	100	150	200	250	300	350	450	550	650	750
Male	0	15	15	14	14	14	14	14	13	12	12	10	9
	10	15	15	15	14	14	14	14	12	12	12	12	8
	50	15	15	15	15	15	15	15	14	14	14	13	10
	100	15	14	14	14	14	13	13	12	12	12	12	11
	500	15	15	14	14	14	14	14	14	14	14	14	13
	1,000	15	15	15	15	15	15	15	12	11	10	10	10
	5,000	15	15	14	14	14	14	14	11	9	9	5	2
	10,000	15	14	14	14	13	13	12	11	10	8	5	2
Female	0	15	15	15	15	15	15	15	15	15	13	12	9
	10	15	15	15	15	15	15	15	15	14	13	8	6
	50	15	13	13	12	12	12	12	11	10	10	6	5
	100	15	15	15	15	15	15	15	14	13	12	11	11
	500	15	15	15	15	15	15	15	12	11	11	9	5
	1,000	15	15	15	15	14	13	13	9	9	7	7	5
	5,000	15	15	15	14	14	14	13	11	11	9	6	5
	10,000	15	15	14	14	13	13	11	9	7	5	3	2

Body weight gain was reduced for the top two concentration groups

MALE BODY WEIGHTS (group mean)

	Days on Test											
Feed, ppm	0	50	100	150	200	250	300	350	450	550	650	750
0	41	247	331	372	386	397	418	435	449	447	449	401
10	41	228	309	357	374	390	406	411	437	449	444	423
50	41	227	303	355	368	376	390	403	418	427	425	378
100	41	236	311	355	370	383	405	423	431	443	433	428
500	42	239	316	357	365	376	387	404	406	410	396	390
1,000	42	239	322	352	366	367	378	395	406	413	407	393
5,000	42	193	261	310	303	320	326	337	322	332	369	367
10,000	42	143	199	223	248	252	260	272	285	286	228	-

FEMALE BODY WEIGHTS (group mean)

	Days on Test											
Feed, ppm	0	50	100	150	200	250	300	350	450	550	650	750
0	41	167	210	244	259	281	287	299	337	360	366	328
10	41	170	217	253	267	282	292	298	321	340	345	375
50	41	162	209	247	260	277	282	294	310	339	356	348
100	41	169	208	244	260	278	283	293	323	348	397	357
500	41	182	210	235	246	260	261	264	300	312	321	313
1,000	40	162	207	235	252	257	252	253	304	337	343	332
5,000	41	143	180	205	218	230	229	239	258	261	257	236
10,000	41	131	152	169	177	187	187	195	202	195	188	-

Organ weights of treated animals at sacrifice were similar to controls except the highest concentration was associated with increased relative kidney weights.

Biphenyl			Mean organ weights /100 g body weight
Conc	#	Body wt	(grams)

(ppm)	rats	(g)	Liver	Kidneys	Heart	Testes
Males						
0	9	396±24.6	2.89±0.16	0.75±0.02	0.32±0.015	0.72±0.03
10	8	424±5.1	2.66±0.06	0.70±0.03	0.28±0.008	0.62±0.07
50	10	383±19.8	2.84±0.15	0.73±0.02	0.30±0.01	0.56±0.06
100	11	394±14.2	2.47±0.07	0.72±0.01	0.31±0.008	0.67±0.07
500	13	371±15.8	3.03±0.12	0.74±0.02	0.31±0.007	0.65±0.06
1,000	10	366±23.7	2.98±0.19	0.83±0.05	0.34±0.012	0.60±0.08
5,000	2	345	3.12	1.17	0.36	0.36
Females						
0	9	333±9.4	3.11±0.15	0.65±0.01	0.33±0.01	
10	6	369±13.4	3.21±0.17	0.62±0.02	0.28±0.07	
50	5	335±16.6	2.81±0.18	0.64±0.02	0.31±0.03	
100	11	341±9.1	3.46±0.74	0.62±0.02	0.30±0.01	
500	5	306±12.5	3.51±0.12	0.68±0.02	0.31±0.01	
1,000	5	327±6.8	3.18±0.10	0.65±0.01	0.32±0.01	
5,000	5	226±25.8	4.52±0.20	1.39±0.14	0.46±0.04	

HISTOPATHOLOGICAL FINDINGS: The only histopathological change that was clearly related to biphenyl consumption occurred in the kidneys. The kidneys of all male and female rats receiving dietary levels of 0.5 or 1.0% biphenyl had prominent irregular scarring, lymphocytic infiltration, tubular atrophy, and patchy tubular dilation to the point of cyst formation. Hemorrhage was present in some dilated tubules and, in some instances, in the renal pelvis. Calculi with basophilic staining foci were frequent it the renal pelvis and similar smaller deposits of precipitated material were sometimes seen in the kidney substance. Some of the dilated tubules contained polymorphonuclear leucocytes and small fragments of nuclear material. Hydronephrosis was common and in several instances there was metaplasia of the epithelium of the renal pelvis to the squamous cell type, but this did not appear to be neoplastic.

Kidneys of female rats on doses of 0.1% or less Biphenyl exhibited no changes that were clearly different from the occasional small scars and focally dilated tubules that were present in the control animals.

In the kidneys from the male animals at all dose levels including the controls, scars and dilated tubules were distinctly more numerous and some degree of hydronephrosis was more prominent than in the females. This corresponded to the observation of deposited material in the renal pelvis or bladder in male animals only, except at the 0.5 and 1.0% feed levels where it was also present in female rats. Most of the kidneys from male rats which received 0.1% or 0.03% Biphenyl similar to controls, except in two of these animals there were masses of partly disintegrated blood in the rectal pelvis and in two others, there were small basophilic concretions in the medullary portions of the kidneys.

Blood was present in the renal pelvis in one animal from each of the other treated groups (0.001, 0.005 and 0.01%). These deposits were sometimes associated with hydronephrosis. Hydronephrosis was also present in several kidneys from the other groups of male animals (including controls) in which pelvic hemorrhage or concretions were not demonstrable. Some of these animals, as well as some others without observed hydronephrosis, presented a protein coagulum in their bladder. This was present in several of the control animals and was clearly unrelated to the treatment. There was a small amount of old blood in the pelvis of one control kidney.

Comparison of the kidneys from the various groups of animals indicated that with doses of 0.1% or less Biphenyl there was no distinct difference from the controls.

No other organ changes could be related to biphenyl ingestion.

PAIRED FEEDING: As records of food consumption revealed a decrease, paired-feeding experiments with rats of each sex receiving 1.0 and 0.5% biphenyl were conducted for 98 days to determine whether the decrease in growth could be accounted for by reduced food consumption. Thirty-eight males and 46 females were used in this paired-feeding experiment. Six weanling rats of each sex were used as ad libitum food consumption controls, 9 males and 10 females were pair-fed based on food consumption at 0.5% Biphenyl, and 7 males and 10 females were pair fed at the 1.0% Biphenyl food consumption level. The mean body weights at the end of the 98-day pair-feeding study were for MALES: ad lib 0% 232 g, ad lib 0.5% 203 g, ad lib 1.0% 172 g; pair-fed control diet at food consumption rate of 0.5% animals, 199g; pair-fed control diet at food consumption rate of 1.0% animals,170g. FEMALES ad lib 0% 150 g, ad lib 0.5% 126 g, ad lib 1.0% 113 g; pair-fed control diet at food consumption rate of 0.5% animals, 123 g; pair-fed control diet at food consumption rate of 1.0% animals, 107g. This indicates that reduced feed consumption and not toxicity was probably responsible for much of the reduced weight gain associated with the groups fed Biphenyl in their diet.

Not shown are reductions in hemoblogin levels measured after 300 days of dosing in the two highest concentrations of dietary biphenyl. Hemoglobin levels were reduced about 30% in the highest concentration but this was attributed to the reduced feed consumption and reduction in weight gain and not a direct effect of the chemical on blood or blood-forming organs.

The incidence of tumors was examined as a function of test substance administration. The incidence of all tumors in treated groups was similar to controls. The number of animals on test, however, would not provide a sensitive bioassay for carcinogenicity.

REPRODUCTIVE TOXICITY TEST:

Two studies of potential reproductive effects and survival of young were conducted. It was concluded that "Dietary levels of 0.1 and 0.5% Biphenyl had no significant effect on reproduction." Please refer to the reproductive toxicity section of this HPV document for details.

Test substance : Biphenyl, CASNO 92-52-4
Conclusion : Feeding dietary levels of 5,000 or 10,000 ppm Biphenyl to rats for up to 750 days was associated with a decrease in weight gain and pathological changes in the kidneys. The reduced body weight gain was attributed to lack of palatability and not a toxic effect of biphenyl. A dietary level of 1000 ppm is considered a NOAEL. In the limited reproductive toxicity test, no effect on reproductive ability or pup survival was found.
Reliability : (2) valid with restrictions
Published reports are assigned a reliability of 2. Despite differences from current protocols, this was a well documented study of considerable scope.
Flag : Critical study for SIDS endpoint
08.02.2005 (25)

Type : Chronic
Species : rat
Sex : male/female
Strain : Fischer 344/DuCrj
Route of admin. : oral feed
Exposure period : 104 weeks
Frequency of treatm. : continuous
Post exposure period : none

Doses	:	500, 1500, 4500 ppm (38, 113, or 338 mg/kg body weight per day)
Control group	:	yes, concurrent vehicle
LOAEL	:	= 500 ppm
Method	:	other: OECD 453 Guideline
Year	:	1981
GLP	:	yes
Test substance	:	

Method :

A chronic study using F344/DuCrj rats was performed according to OECD 453 guideline. Groups of 50 male and 50 female rats were given the control diet or the biphenyl-containing diets throughout the 105-week period, starting at the age of 6 weeks. Dietary concentrations of biphenyl were 500, 1500 or 4500 ppm (38, 113, or 338 mg/kg body weight per day).

Body weight and food consumption were measured once a week for the first 14 week of the 105-week study period and every 4 week thereafter. Urinary parameters of all surviving rats, including pH and occult blood, were examined with Urolabsix (Diagnostic Diutsior, Bayer, Elkhard, Germany) in the final week of the 105 week study period. All organs were examined macroscopically, selected organs, including gonads, weighed and the tissues for microscopic examination included the ones specified in the OECD test guidelines, and were fixed in 10% neutral buffered formalin, embedded in paraffin and 5um thick sections of all tissues and tumors were made and stained with hematoxylin and eosin.

All organs and tissues were preserved for microscopic examination. Based on the guideline (not defined in the publication) this included the following organs and tissues: brain* (medulla/pons, cerebellar cortex, cerebral cortex), pituitary, thyroid (including parathyroid), thymus, lungs (including trachea), heart, salivary glands, liver*, spleen, kidneys*, adrenals*, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes, pancreas, gonads*, uterus, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord (cervical, thoracic, lumbar), sternum with bone marrow and femur (including joint) and eyes.

The incidence of non-neoplastic lesions and the urinary data were analyzed with the Chi-square test.

* Organs from 10 animals/sex/dose level for rodents were weighed.

Test substance : Biphenyl, CASNO 92-52-4, with a purity of >98% was obtained from Wako Pure Chemical Industries, Tokyo, Japan.

Result : Clinical Observations:
Body weights of rats fed 4500 ppm biphenyl in the diet were significantly decreased compared to control values but there was no statistical difference in the body weight between the 500 or 1500 ppm rats of both sexes and the corresponding controls (Fig 1).

Surival rates of all biphenyl-exposed groups except the 4500 ppm males were not statistically different from those of corresponding controls (Fig 2). Nineteen 4500 ppm males died during the 105-wk period. Their deaths were attributed primarily to the bladder tumors and the hematuria.

Thirty two males with clinical hematuria were observed and of the 32 males, 14 had anemia-colored skin and/or eyes in the 4500 ppm group. The hematuria first appeared around the 40th week of biphenyl exposure and continued thereafter with intermittent recoveries. The biphenyl exposed females had no clinical signs relating to biphenyl exposure.

Urinalysis:
The urinary pH significantly increased in the 4500 ppm males (Table 1). The incidence of positive occult blood significantly increased in the 4500

ppm rats of both sexes, and this was consistent with the above mentioned incidence of hematuria in the 4500 ppm males, but the number of females with positive occult blood was smaller than that of the males.

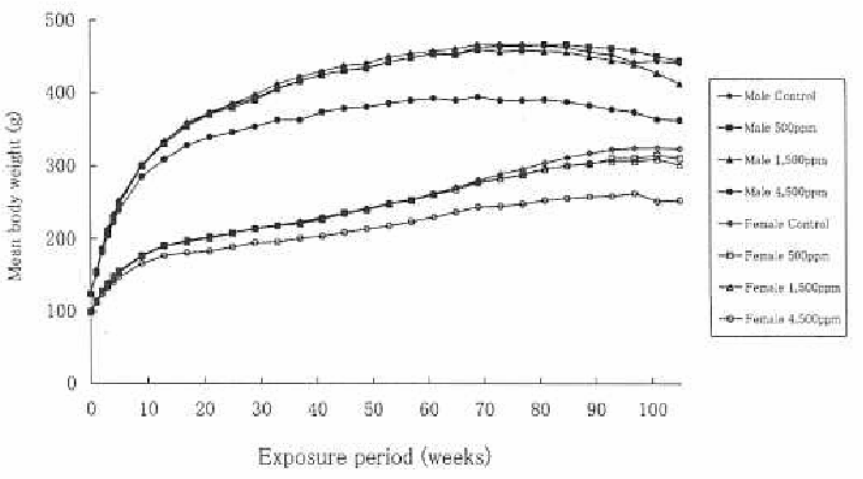
Organ weight:
A statistically significant increase in relative kidney weight was evident in the 1500 and 4500 ppm rats of both sexes and absolute kidney weight significantly increased in the 4500 ppm males (data not shown in publication).

Gross findings:
Bladder calculus was formed predominantly in male rats (Table 2): Forty three 4500 ppm males had bladder calculi, whereas only eight 4500 ppm females had calculi. No bladder calculus was found in any rats of either sex exposed to 500 or 1500 ppm biphenyl. Necropsy of dead and moribund animals revealed bladder calculi first appeared around the 40th week of the exposure together with the occurrence of hematuria.

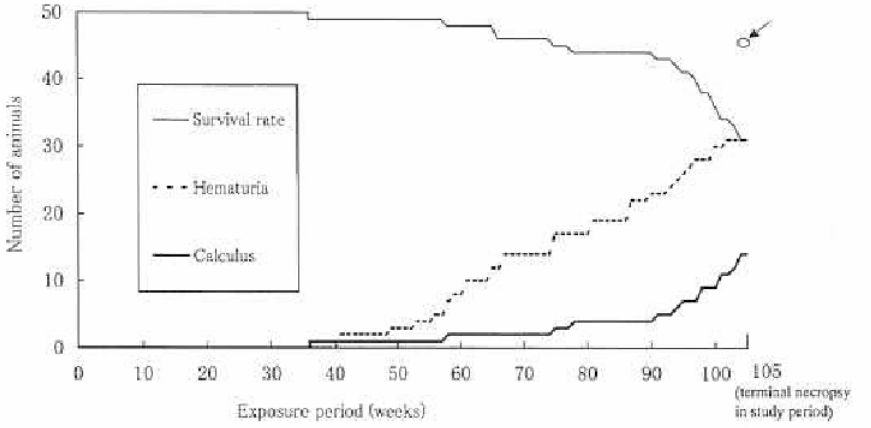
Histopathologic findings:
Non-neoplastic lesions were limited to the urinary tract (Tables 2 and 3). Transitional cell hyperplasia, squamous cell hyperplasia and squamous cell metaplasia were observed in the urinary bladder of the 4500 ppm group. Hyperplasias were not diffusely distributed over the entire area of the bladder epithelium but developed in the focal area. The transitional cell hyperplasia was further classified into simple, nodular and papillary hyperplasia according to the histologic proliferation patterns by IARC and the Standardized System of Nomenclature and Diagnostics Criteria. The incidences of simple, nodular and papillary hyperplasia were 24%, 80% and 24% in the 4500 ppm males and 2%, 10% and 8% in the 4500 ppm females, respectively, indicating that the simple hyperplasia occurred less frequently than the nodular and papillary hyperplasias. The simple hyperplasia was almost always accompanied by either nodular or papillary hyperplasia in the males. Ten 4500 ppm males had polyps in the bladder epithelium. The polyps observed in the present study were composed of abundant spindle cells that were proliferated around the transitional epithelial cells accompanied by inflammatory infiltration of the submucosal bladder epithelium, and were classified as the inflammatory type according to the Pathology of the Fischer rat. The polyps were accompanied by squamous metaplasia on their surface, and found at different loci from the bladder tumors.

In the ureters, the incidences of simple transitional cell hyperplasia and dilatation lumen were greater in the 4500 ppm males than in the corresponding females. In the renal pelvis, simple and nodular hyperplasia occurred frequently not only in the 4500 ppm males but also in the females exposed to 1500 and 4500 ppm. In the kidneys, statistically increased incidences of mineralization of cortico-medullary junction in the 4500 ppm males and mineralization of papilla in the 4500 ppm males and females were noted, whereas papillary necrosis, infarct and hemosiderin deposition occurred predominantly in the females.

Attached document : Figure 1 Time-course changes in mean body weight of male or female F344 rats exposed to 0, 500, 1500 or 4500 ppm biphenyl in the diet for 105 wk



Attached document : Figure 2 Time-course changes in the survival rate, the hematuria and the bladder calculi of the 4500 ppm males. The number of dead or moribund and necropsied animals with bladder calculi are indicated by a solid line and an open circle with arrow, respectively. The dotted line indicates cumulative number of animals showing hematuria



Attached document : Table 1. Urinalysis results of male and female F-344 rats exposed to 0, 500, 1,500 or 4,500 ppm biphenyl in the diet for 105 wk

Sex	Group name	No. of animals	Average urinary pH	Positive occult blood[a]
Male	Control	37	7.66 ± 0.49	1
	500 ppm	41	7.67 ± 0.40	1
	1,500 ppm	41	7.57 ± 0.57	2
	4,500 ppm	31	7.97 ± 0.43*	23**
Female	Control	45	7.29 ± 0.69	1
	500 ppm	38	7.11 ± 0.64	0
	1,500 ppm	45	7.24 ± 0.65	0
	4,500 ppm	37	7.26 ± 0.60	10*

Attached document : Table 2 Incidences of urinary bladder lesions in male and female F-344 rats exposed to 0, 500, 1500 or 4500 ppm biphenyl in the diet for 105 wk

Sex of animal	Male				Female			
Group and the dose of chemicals administrated (per os)	Control	500 ppm	1,500 ppm	4,500 ppm	Control	500 ppm	1,500 ppm	4,500 ppm
Number of rats examined	50	50	50	50	50	50	50	50
transitional cell hyperplasia								
simple hyperplasia[a]	0	0	0	12[c]	0	0	1	1
nodular hyperplasia[a]	0	0	0	40[c]	1	0	0	5
papillary hyperplasia[a]	0	0	0	17[c]	0	0	0	4
total transitional cell hyperplasia	0	0	0	45	1	0	1	10
transitional cell papilloma	0	0	0	10[b,d]	0	0	0	0
transitional cell carcinoma	0	0	0	24[b,d]	0	0	0	0
total number of bladder tumors	0	0	0	31	0	0	0	0
squamous metaplasia[a]	0	0	0	19[c]	0	0	0	4
squamous cell hyperplasia[a]	0	0	0	13[c]	0	0	0	1
squamous cell papilloma and carcinoma	0	0	0	1	0	0	0	0
inflammatory polyp[a]	0	0	0	10[c]	0	0	0	0
calculus[a]	0	0	0	43	0	0	0	8

Attached document : Table 3 Incidences of ureter and kidney lesions in male and female F-344 rats exposed to 0, 500, 1500 or 4500 ppm biphenyl in the diet for 105 wk

Sex of animal	Male				Female			
Group and the dose of chemicals administrated (per os)	Control	500 ppm	1,500 ppm	4,500 ppm	Control	500 ppm	1,500 ppm	4,500 ppm
Number of rats examined	50	50	50	50	50	50	50	50
ureter								
transitional cell hyperplasia								
simple hyperplasia	1	0	0	8**	0	0	0	2
nodular hyperplasia	0	0	0	1	0	0	0	0
dilatation	0	0	0	14**	0	0	0	6
kidney								
renal pervis								
transitional cell hyperplasia of renal pelvis								
simple hyperplasia	6	8	5	19*	3	5	12*	25**
nodular hyperplasia	0	1	1	21**	0	0	1	12**
squamous metaplasia	0	0	0	2	0	0	0	0
mineralization of pelvis	9	6	10	18	12	12	18	27**
desquamation:pelvis	1	0	0	11**	0	0	0	2
calculus	0	0	0	13**	0	0	0	3
other								
mineralization of cortico-medullary junction	0	0	0	10**	21	2	26	18
mineralization of papilla	9	9	14	23*	2	6	3	12**
papillary necrosis	0	0	0	7	0	0	0	23**
infarct	0	0	0	0	1	0	0	8*
deposit of hemosiderin	0	0	0	0	4	8	22**	25**
chronic nephropathy	45	45	43	34	33	35	30	26

Conclusion : Summary of Effects:

High dose: Bladder hyperplasia in males and females, kidney hyperplasia and mineralization in males and females, clinical chemical and hematological changes in males and females, increase in urea nitrogen in males and females.

Mid dose: Kidney mineralization in males and females (minimal), increase in urea nitrogen (males and females), clinical chemical and hematological changes (males and females).

Low dose: Increase in urea nitrogen (males), clinical chemical and hematological changes (males)

Remark : Although the CICAD refers to clinical chemistry and hematological changes in males and females, there is no such indication in the Umeda et al., 2002 paper.

Reliability : (1) valid without restriction
GLP guideline study

01.03.2005 (26)

Type : Chronic
Species : mouse
Sex : male/female
Strain : other: Crj:BDF1
Route of admin. : oral feed

Exposure period	:	104 weeks
Frequency of treatm.	:	continuous
Post exposure period	:	none
Doses	:	100, 300, 900 mg/kg-day
Control group	:	yes, concurrent vehicle
Method	:	other: OECD 453 Guideline
Year	:	1981
GLP	:	yes
Test substance	:	

Method :

A chronic study using Crj:BDF1 mice of each sex was performed according to standard protocols. Groups of 50 mice of each sex were given diets containing 0, 667, 2000, or 6000 mg biphenyl/kg/day in the diet (corresponding to 0, 97, 291, or 1050 mg/kg body weight per day in males and 0, 134, 414 or 1420 mg/kg body weight/day in females) for 104 weeks. At the end of the dosing period surviving mice were fasted overnight, blood samples collected for hematology and urinalysis determinations. The animals were sacrificed, examined for gross effects, selected organs were removed and weighed, tissues were removed, fixed, sectioned, stained with H&E and examined for microscopic changes.

All organs and tissues were preserved for microscopic examination. Based on the guideline (not defined in the publication) this included the following organs and tissues: brain* (medulla/pons, cerebellar cortex, cerebral cortex), pituitary, thyroid (including parathyroid), thymus, lungs (including trachea), heart, salivary glands, liver*, spleen, kidneys*, adrenals*, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes, pancreas, gonads*, uterus, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord (cervical, thoracic, lumbar), sternum with bone marrow and femur (including joint) and eyes.

Statistical analysis: Incidences of non-neoplastic lesions were analyzed by Fisher's exact test. Incidences of neoplastic lesions were statistically analyzed by Peto's trend test and Fisher's exact test. Body weight, food consumption, organ weight and hematological and blood biochemical parameters were analyzed by Dunnett's test.

* Organs from 10 animals/sex/dose level for rodents were weighed.

Result :

Survival, body weights, food consumption and clinical signs: Body weights of the males and females fed 6000 ppm in the diet were significantly lower than the respective control (Fig 1). Food consumption of any biphenyl-fed group of either sex was not decreased throughout the 2-year administration period, compared with the respective control (Table 1). There was no difference in survival rate between any biphenyl-fed group of either sex and the respective control (Fig 2). No overt clinical signs were observed in any biphenyl-fed group.

Hematology and blood biochemistry: There was no significant difference in any hematological parameter between any biphenyl-fed group of either sex and the respective control. Blood urea nitrogran (BUN) was increased slightly in males fed 2000 or 6000 ppm in the diet and in the females fed 6000 ppm in the diet (Table 2). Alkaline phosphatase (ALP) activity was increased in the males and females fed 6000 ppm in the diet. Although no significant change in glutamic oxalacetic transaminase (GOT) or glutamic pyruvic transaminase (GPT) activity was found in any of the males fed diets containing biphenyl, dose-dependent increases in the serum levels of the transaminases were noted in the females fed 2000 or 6000 ppm in the diet. Lactate dehydrogenase (LDH) activity was also increased in the females fed diets containing 2000 or 6000 ppm.

Organ weights and gross findings: No significant difference in organ

weight between any biphenyl-fed group of either sex and the respective control was found except for relative liver weights of females. Relative liver weights of the females fed 667, 2000 and 6000 ppm diets were increased 1.3-, 1.4- and 1.6-fold, respectively, compared with the female controls.

Incidences of liver nodules in the females were increased in a dose-related manner, whereas those in the males were not (Table 3). The nodules were round-or oval-shaped and cystic or solid mass, with the diameter of major axis varying from 3 to 23 mm.

Histopathological examination: Histopathological observations were confined primarily to the liver and kidney. Incidence of basophilic cell foci was significantly increased in females fed 2000 or 6000 ppm in the diet. Although the incidences of basophilic cell foci (12%) and clear cell foci (12%) were significantly increased in the males fed 667 ppm in the diet, the incidences of those pre-neoplastic lesions were not dose-related.

Incidences of desquamation of the urothelium in the renal pelvis were significantly increased in the males and females fed 6000 ppm in the diet. The necrotic urothelium accompanied by mineralization was found in the renal pelvic cavity. Incidences of the mineralization in the inner stripe of outer medulla of the kidney were significantly increased in females fed 2000 or 6000 ppm in the diet.

Attached document :

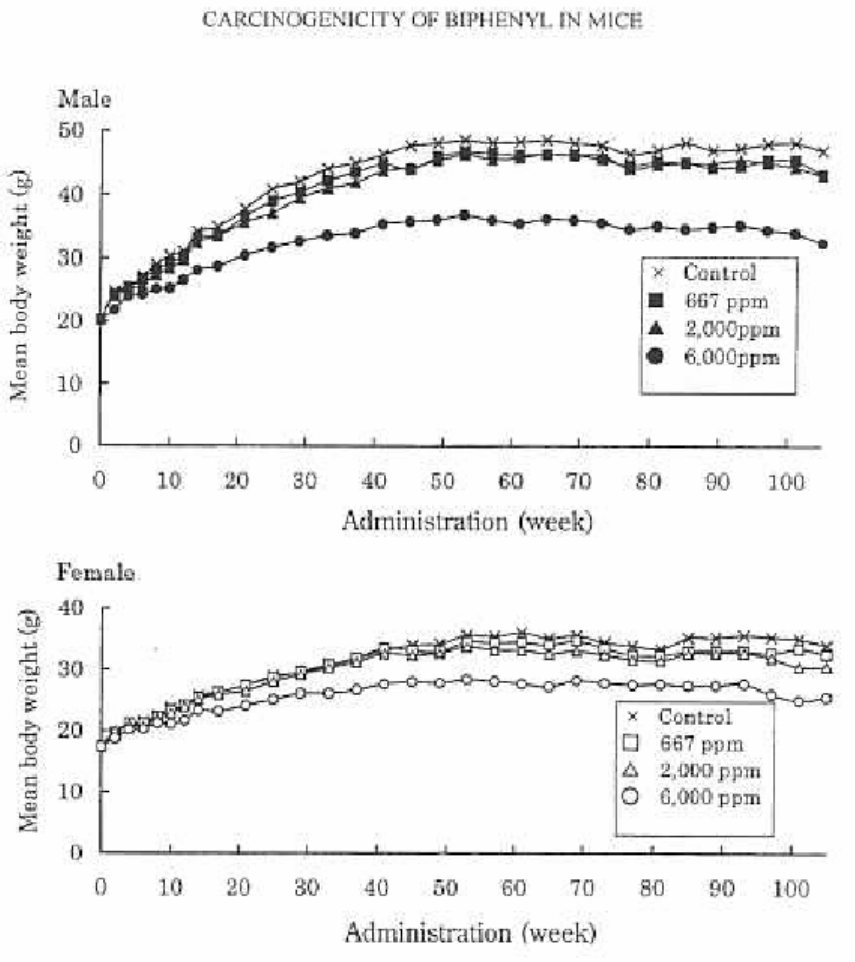


Fig. 1. Time-course changes in the mean body weights of the male or female BDF_1 mice fed diets containing biphenyl at 667, 2,000 or 6,000 ppm for two years.

Attached document :

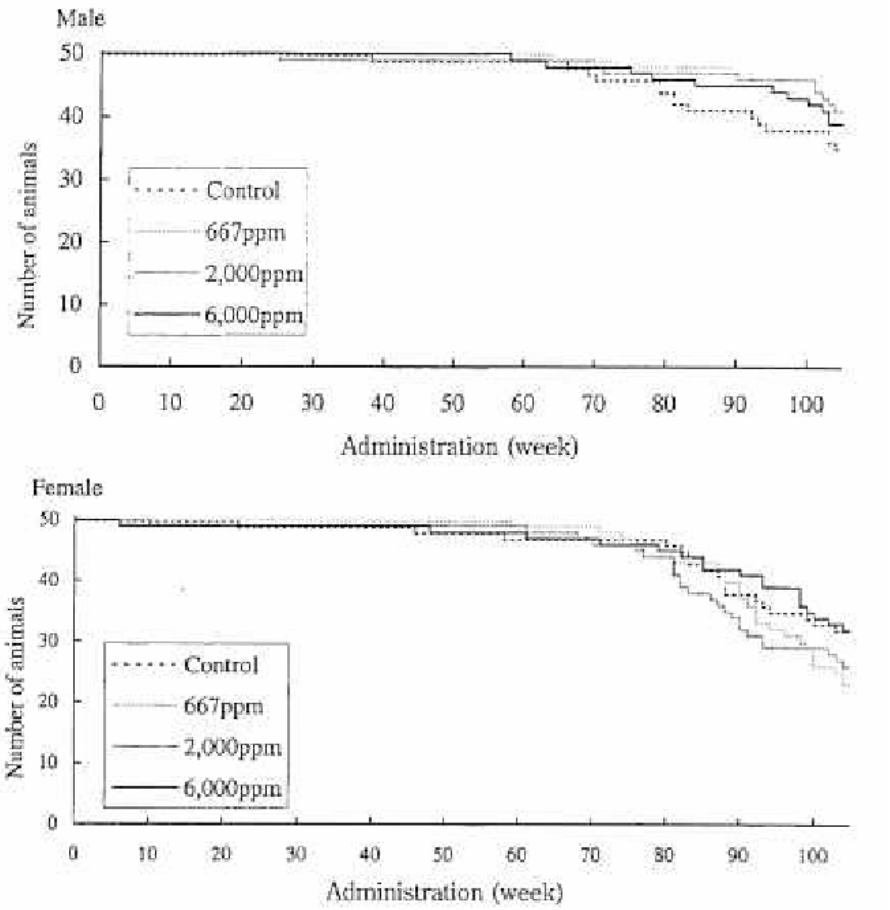


Fig. 2. Time-course changes in the survival rates of the male or female BDF_1 mice fed diets containing biphenyl at 667, 2,000 or 6,000 ppm for two years.

Attached document :

Table 1. Survival rate, body weight, food consumption and daily biphenyl intake of the mice fed diets containing biphenyl for two years

Biphenyl concentration in diet (ppm)	Survival rate at the end of 2-yr administration period	Body weight at the end of 2-yr administration period[a)] (g)	Averaged food consumption[b)] (g/day)	Daily biphenyl intake[b)] (mg/kg)
Male				
Control	35/50	46.9 ± 4.9	5.6	0
667	41/50	43.1 ± 7.9	5.5	97
2,000	41/50	42.9 ± 6.0*	5.5	291
6,000	39/50	32.4 ± 3.6**	5.4	1,050
Female				
Control	31/50	34.0 ± 4.0	5.9	0
667	22/50	32.5 ± 3.3	5.8	134
2,000	25/50	30.5 ± 3.1**	5.9	414
6,000	32/49	25.5 ± 3.0**	5.9	1,420

* and **: Significantly different at $p<0.05$ and $p<0.01$, respectively ,by Dunnett's test.
a) Values of body weight were expressed as mean ± standard deviation.
b) Food consumption and biphenyl intake were averaged over the 2-yr administration period.

Attached document :

Table 2. Blood biochemistry in the male and female mice fed diets containing biphenyl for two years

Group Name	Male				Female			
	Control	667 ppm	2,000 ppm	6,000 ppm	Control	667 ppm	2,000 ppm	6,000 ppm
No. of Animals	34	39	37	37	28	20	22	31
GOT (IU/l)	85 ± 92	58 ± 38	69 ± 60	88 ± 151	75 ± 27	120 ± 110	211 ± 373**	325 ± 448**
GPT (IU/l)	73 ± 113	34 ± 31	36 ± 49	43 ± 80	32 ± 18	56 ± 46	134 ± 231**	206 ± 280**
ALP (IU/l)	178 ± 111	155 ± 30	169 ± 36	261 ± 102**	242 ± 90	256 ± 121	428 ± 499	556 ± 228**
LDH (IU/l)	321 ± 230	252 ± 126	432 ± 868	283 ± 200	268 ± 98	461 ± 452	838 ± 2,000	1,416 ± 4,161*
BUN (mg/dl)	20.2 ± 3.6	22.0 ± 4.0	23.2 ± 4.4*	22.9 ± 2.7**	14.9 ± 2.0	14.8 ± 3.4	21.0 ± 20.5	23.8 ± 11.7**
Calcium (mg/dl)	9.2 ± 0.6	9.0 ± 0.5	9.1 ± 0.5	9.2 ± 0.3	9.0 ± 0.2	9.1 ± 0.4	9.5 ± 0.7**	9.6 ± 1.1**
Sodium (mEq/l)	152 ± 1	153 ± 2	153 ± 2	155 ± 2**	152 ± 2	152 ± 2	152 ± 3	155 ± 4**
Potassium (mEq/l)	4.4 ± 0.4	4.2 ± 0.4	4.2 ± 0.4	4.1 ± 0.3**	4.1 ± 0.3	4.3 ± 0.4	4.1 ± 0.7	4.0 ± 0.5
Chloride (mEq/l)	122 ± 3	124 ± 3	124 ± 2*	125 ± 3**	125 ± 3	124 ± 3	122 ± 6	124 ± 5

Values were expressed as mean ± standard deviation.
* and **: Significantly difference at $P<0.05$ and $P<0.01$, respectively, by Dunnett's test.
GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvic transaminase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, BUN: blood urea nitrogen.

Attached document :

Table 3. Incidences of gross and histopathological findings in the male and female mice fed diet containing biphenyl for two years

Sex of animal	Male				Peto's	Female				Peto's
Group (ppm)	Control	667	2,000	6,000	test	Control	667	2,000	6,000	test
Number of mice examined	50	49	50	50		50	50	50	49	
Gross finding										
Liver										
Nodule	20	16	14	11		7 (5)[c]	13 (5)	24 (16)	26 (19)	
Histopathological findings										
Liver										
Hepatocellular adenoma	8	6	7	3		2	3	12*	10*	↑
Hepatocellular carcinoma	8	8	5	4		1	5	7*	5	
Hepatocellular adenoma + carcinoma[a]	16	12	9	7		3	8	16**	14*	↑↑
Basophilic cell foci[b]	0	6**	1	2		1	1	12**	6*	
Clear cell foci[b]	0	6**	2	0		2	1	3	2	
Eosinophilic cell foci[b]	0	0	0	0		0	1	0	0	
Kidney										
Desquamation: pelvis[b]	0	0	0	10**		4	0	0	15**	
Mineralization in the inner stripe-outer medulla[b]	9	8	14	14		3	5	12*	26**	

* and **: Significantly different at $p<0.05$ and $p<0.01$, respectively, by Fisher's test.
↑ and ↑↑: Significantly different at $p<0.05$ and $p<0.01$, respectively, by Peto's test.
a) Combined incidence of hepatocellular adenoma and carcinoma.
b) Number of the histopathological finding with a different grade (slight, moderate, marked or severe) was summed.
c) The parenthesized value indicates the number of the animals bearing the liver nodule in which the proliferative lesion was histopathologically observed.

Test substance : Biphenyl, CAS# 92-52-4. Purity greater than 98% was obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan.

Remark : Body weights of male and female mice fed 6000 ppm in the diet were decreased 30 and 25%, respectively. The degree of body weight effects observed clearly exceed the maximum tolerated dose.

As discussed by Umeda et al., 2004, peroxisome proliferation was observed in the liver of female mice fed 16,000 ppm biphenyl for 13 weeks. This was not observed in male mice in the same study.

In a subacute study, Sunouchi et al., 1999, reported slight increases in KCN-insensitive palmitoyl CoA oxidation in liver homogenates and lauric acid 12-hydroxylation in liver microsomes in animals receiving 5.2 mmol/kg biphenyl (~800 mg/kg). The response observed was much lower than for the positive control, clofibrate, which is generally considered a weak to moderate peroxisome proliferator. The dose levels used by Sunouchi were much lower than those used in the chronic study, thus it is unclear the extent of peroxisome proliferation that occurred at the higher doses.

In personal communication with Dr Umeda, the absolute and relative liver weights for the various dose levels in the two year study were as follows:

Table 1. Absolute and relative liver weights of both males and females in the 2-year study

Group (ppm)	Control	667	2000	6000
Male				
No. of animals[a]	35	41	41	39
Body weight[b]	44.7 ± 5.1	41.2 ± 7.5	41.0 ± 5.9	30.6 ± 3.4
Absolute (g)	1.682 ± 0.800	1.562 ± 0.709	1.617 ± 0.842	1.332 ± 0.232
Relative (%)	3.899 ± 2.325	4.015 ± 2.581	4.035 ± 2.277	4.388 ± 0.881
Female				
No. of animals[a]	31	22	25	32
Body weight[b]	32.0 ± 3.9	31.1 ± 3.2	28.1 ± 2.9	23.8 ± 2.7
Absolute (g)	1.271 ± 0.197	1.590 ± 0.638	1.530 ± 0.637	1.509 ± 0.587
Relative (%)	4.022 ± 0.755	5.216 ± 2.282	5.505 ± 2.374	6.482 ± 2.900

Mean±S.D.
a: The values indicate number of animals surviving to the end of 2-year administration period
b: The data indicate the mean body weights in survival animals which fasted overnight after at the end of 2-year administration period

The relative liver weights in male mice ingesting 6000 ppm group was increased approximately 12% from control values while the same parameter in female mice was increased 60%.

The authors suggest peroxisome proliferation is induced by the formation of 2,5-dihydroxybiphenyl which is structurally similar to CI-924 a peroxisome proliferator. The presumption by the authors is that the first metabolite formed by the degradation of biphenyl is 2-hydroxybiphenyl. However, as

reviewed by Bomhard et al., (2002), in chronic studies of 2-hydroxybiphenyl (also known as orthophenylphenol) peroxisome proliferation was not reported in male or female mice. Thus it is unclear what induces peroxysome proliferation.

Reference
Bomhard, E.M., Brendler-Schwaab, S.Y., Freyberger, A., Herbold, B.A., Leser, K.H. and Richter, M. (2002). Critical Reviews in Toxicology 32:551-626.

Reliability : (1) valid without restriction
Modern guideline study under GLP's with sufficient documentation

01.03.2005 (27)

Type	:	Sub-chronic
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	inhalation
Exposure period	:	13 weeks
Frequency of treatm.	:	7 hours/day 5 day/wk
Post exposure period	:	30 days
Doses	:	25 and 50 ppm
Control group	:	yes, concurrent vehicle
LOAEL	:	= 25 ppm
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method : A 13-week vapor inhalation study using groups of 50 CD-1 mice of each sex exposed to 25 or 50 ppm (160 or 320 mg/m3; analytical concentrations) biphenyl for 7 hours/day, 5 days/week was conducted. Mice were obtained as weanlings (5-25 grams) and received food and water ad libitum except during the 7-hour exposures.

Exposures were conducted in 0.5 cubic meter stainless steel "Rochester" type chambers with glass windows on all four sides for viewing. During the exposures, 10 mice of one sex were housed in a cage with a divider such that 5 mice were together on one side of the cage. Cages were placed on a raised wire mesh floor in the exposure chamber.

Biphenyl vapor was generated by heating biphenyl, contained in a three-necked flask, in an oil-bath while directing air in one of the necks and out another through a heated connector tube to the chamber. Airflow was maintained at 2 L per minute. The concentration of test material in the chambers was determined twice daily by drawing a known volume of vapor through two impingers in tandem containing cyclohexane. The solutions were analyzed for biphenyl by uv against a standard curve.

Mice were observed during the exposure for adverse clinical signs and were weighed weekly. Near the end of the study, each group of surviving mice was placed in a metabolism cage for a 12-hour urine collection. Blood for hematology was collected at sacrifice from the dorsal vein after opening the pleural cavity.

Ten mice of each sex from each group were held for a 30-day recovery period prior to sacrifice.

Some difficulties occurred maintaining the biphenyl level during the first weeks of exposure but these issues were solved and exposure control was tighter during the remainder of the study. The average concentration of biphenyl in the chambers was 25 ± 7 ppm (26.5 ± 1 ppm during the last 72

days) and 50 ± 16 ppm (51.4 ± 9.6 ppm during the last 55 days).

Due to a technical problem several mice in the 25-ppm exposure group were inadvertently killed at week 12 of the study when they were overheated in a holding room. These mice were replaced with unexposed weanling mice and the entire group was exposed until the replacement mice had received 65 exposures.

At study termination all surviving animals were submitted to a gross examination and tissues of the following organs were collected, prepared and microscopically examined: trachea, lungs, livers, kidney and spleens.

Result **:** Exposed mice weight gain was comparable to controls throughout the study. An explicit table giving mortality was not included in the report. Due to the inclusion of the replacement mice and the poor legibility of the tables the mortality could not be determined from the tables of individual animal weights. Mortality per group could not be reliably ascertained from the pathology report, which noted that only 71 high-dose animals and 98 low-dose animals were available for examination at study termination. This suggests some compound-related mortality in the high-dose group but the extent cannot be verified.

Clinical chemistry parameters were SGOT, SGPT, alkaline phosphatase, bilirubin, uric acid and BUN. Statistical analysis was not presented in the report and, due to poor legibility, post-hoc analysis was impossible. Examination of the results suggests that SGOT and SGPT were elevated in high-dose animals of each sex sacrificed at the end of 13-weeks exposure while all other parameters were unremarkable. Not enough blood was obtained from the 25-ppm males to allow clinical chemistry evaluations. Clinical chemical determinations were conducted after the 30-day recovery period but only on two animals per group. In these 4 (2 of each sex) 50-ppm animals, the SGPT and SGOT levels were similar to controls.

Except for a possible increase in white blood cells in 25-ppm group females at the end of the exposure period, hematology values were unremarkable. Blood from all 25-ppm males was hemolized and no data were recorded for this group. Hematology was also conducted on two mice from each 30-day recovery group and the results were unremarkable; however, with the limited sample, no conclusions can be drawn.

At gross examination, a finding of congested lungs or lungs hemorrhagic were reported in the majority of high-dose animals, about half of the low-dose animals and about 10 percent of control animals. With the exception of sporadic findings of "small spleen", no gross changes were recorded except for the lungs.

Microscopic examination resulted in a diagnosis of hyperplasia with inflammation of the trachea for 70/71 high-dose animals, 80/89 low-dose animals and 0/80 controls. After 30 days of recovery, the incidence of hyperplasia with inflammation of the trachea was 5/19 at the high dose, 2/15 at the low dose and 3/20 in controls. Congestion of the lungs, liver and kidneys observed in several animals at microscopic examination was attributed by the pathologist to an effect of the anesthetic used for sacrifice. Congestion and edema of the lungs was found with incidence similar to hyperplasia of the trachea; however, based on the pathologists remark about the congestion being related to anesthetic administration at sacrifice, it cannot be determined if this was compound related.

Remark **:** The pathologist considered the congestion observed in the lung, liver and kidneys of rats exposed to 50 ppm to be due to the anesthetic used. The incidence of congestion for these three tissues was 100% in rats exposed to 50 ppm. In the animals exposed to 25 ppm, the incidence of congestion was slightly less, ranging from 89 to 97% in the three tissues. There was no evidence of congestion observed in the lung, liver and kidneys from

control animals. Thus there appears to be a difference in the amount of congestion observed between control and treated animals with a slight difference observed between the low and high dose animals. Based on the observed response, the conclusions of the pathologist appear to be inconsistent with the data.

Test substance : Biphenyl, CASNO 92-52-4, purity 99%

Conclusion : Inhalation of biphenyl vapor for 13-weeks results in marked respiratory tract inflammation and hyperplasia of the trachea in mice of each sex at 50 ppm with 25 ppm being a LOAEL. The effects appear to be partially reversible after a 30-day recovery period. A NOAEL was not identified

Reliability : (4) not assignable
Due to limited scope and technical difficulties the overall study is assigned a reliability of 4; however, the histopathological examination and reporting of findings to the trachea is considered to have a higher reliability.

01.03.2005 (28)

Type : Sub-chronic
Species : rat
Sex : male
Strain : Fischer 344/DuCrj
Route of admin. : oral feed
Exposure period : 13 weeks
Frequency of treatm. : daily
Post exposure period :
Doses :
Control group :

Method : Groups of five male F344/DuCrj rats (Charles River Japan, Inc., Atsugi, Japan) were fed the control diet (CONTROL) or a diet containing 1.6% biphenyl (BP), 1.6% biphenyl plus 5% KHCO3 (BP + KHCO3), 1.6% biphenyl plus 5% KCl (BP + KCl), 1.6% biphenyl plus 8% NaHCO3 (BP + NaHCO3, 5% KHCO3 (Control + KHCO3) or 5% KCl (CONTROL + KCl) for 13 weeks.

Individual body weights were recorded weekly for the 13 weeks of the study. Necropsy was performed on each animal under ether anesthesia at 13 weeks; the kidney and ureter were fixed in 10% neutral buffered formalin.

Urine sampling and pH, potassium and sodium measurement:
Urine samples were collected individually from each of the five rats fed the chemical-containing diets, housed in polycarbonate metabolic cages for 24 hours on the last day of the experiment. The pH of freshly voided urine was measured in morning samples on the last day of the experiment with a model HN-21P pH meter (Toa Electronic Ltd., Tokyo, Japan). The contrations of potassium and sodium in urine samples collected with metabolic cages were analyzed using a 7070 Automatic Analyzer (Hitachi Ltd., Tokyo, Japan).

Sample preparation for analyses of urine, plasma and ureter contents:
Precipitates were removed from the urine samples (2 ml) by centrifugation, and the supernatants were mixed wih 2 ml acetonitrile. The precipitates were dissolved in 1 ml of acetonitrile, centrifuges and the supernatant was evaporated under vacuum at 40C to obtain the urine crystals. Blood samples (5 ml) collected into test tubes containing heparin and lithium were centrifuged, and the plasma (0.1 ml) was mixed with 0.9 ml acetonitrile. These acetonitrile solutions (0.1 ml) were diluted with 0.9 ml of water and used for LC-MS/MS analysis of the biphenyl sulfate conjugates.

Biphenyl, various hydroxy biphenyls, KHCO3, KCl and NaHCO3 were obtained from Wako Pure Chemical Ind. (Osaka, Japan), Kanto Chemical

Co., Inc. (Tokyo, Japan) or Tokyl Kasei Kogyo Co., Ltd. (Tokyo, Japan). No purity information was supplied. The biphenyls included 2-hydroxy-biphenyl, 3-hydroxy-biphenyl, 4-hydroxy-biphenyl, 2,3-dihydroxy-biphenyl, 4,4'-dihydroxy-biphenyl, and 2,2'-dihydroxy-biphenyl.

Various hydroxy-biphenyl-O-sulfate conjugates were synthesized according to the methods of Hoiberg and Mumma (1969) and Hardy and Scalera (1952). These included 2-hydroxy-biphenyl-O-sulfate (2-HBPOS), 3-hydroxy-biphenyl-O-sulfate (3-HBPOS),
4-hydroxy-biphenyl-O-sulfate potassium salt (4-HBPOSK),
2,3-dihydroxy-biphenyl-O-sulfate (2,3-DHBPOS),
3,4-dihydroxy-biphenyl-3-O-sulfate (3,4-DHBP-3-OS),
3,4-dihydroxy-biphenyl-4-O-sulfate (3,4-DHBP-4-OS),
4,4'-dihydroxy-biphenyl-O-sulfate (4,4'-DHBPOS), 2,5-dihydroxy-biphenyl-O-sulfate (2,5-DHBPOS), 2,2'-dihydroxy-biphenyl-O-sulfate (2,2-DHBPOS),

Solubility with 4-HBPOSK in urine or plasma:
A purified sample of synthetic 4-HBPOSK was added to either mixed urine or plasma collected from five rats for each of CONTROL, CONTROL + KHCO3 and CONTROL + KCl to a concentration of 10 mg/ml and emulsified by ultrasonication for 30 min at 37C. The suspension was filtered through a 0.45 um membrane filter, and the filtered solution was mixed with acetonitrile. The acetonitrile solution was diluted with water and used for LC-MS/MS analysis.

LC-MS/MS chromatography:
Biphenyl sulfate conjugates in urine and plasma and the standard samples were separated by LC-MS/MS. LC-MS/MS analysis was performed using a model 1090 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) interfaced with a TSQ 7000 triple-quadrupole mass spectrometer (Thermoquest). A TSK-Gel ODS-80TS column (Tosoh, Tokyo, Japan) (150 x 2.0 mm id) at a flow rate of 0.25 ml/min, with the column at ambient temperature. The mobile phase was acetonitrile-5 mM ammonium acetate (23:77, v/v).

Statistical analysis:
The significance of the difference between values was estimated by Student's t test. Results are expressed as means +/- SD and were considered statistically significant at $p<0.05$.

References:
Hardy, W.B. and Scalera, M. (1952). Separation of mixtures with triethylamine-sulfur trioxide. J. Am. Chem. Soc. 74:5212-5214.

Hoiberg, C.P. and Mumma, R.O. (1969). Preparaton of sulfate esters. Reactions of various alcohols, phenols, amines, mercaptans, and oximes with sulfuric acid and dicyclohexylcarbodiimide. J. Am Chem Soc 91:4273-4278.

Test substance : Biphenyl was obtained from Wako Pure Chemical Ind (Osaka, Japan). No purity information was provided. However, biphenyl obtained from the same source was used in the 104 week rat study with a purity of >98%.

Result : The body weights at week 13 were markedly reduced in BP + KHCO3 compared to those values for the other groups, consistent with the reduction in feed consumption (Table 1). Hydronephrosis and bleeding in urine were observed in the kidneys in BP + KHCO3 only, but not in CONTROL, BP, PB + KCl, BP +NaHCO3, CONTROL + KHCO3 and CONTROL +KCl. Reduction of the body weight in BP + KHCO3 correlated with the incidence of hydronephrosis. Only KHCO3 enhanced the effects of biphenyl with respect to body weight and feed consumption. However, KHCO3 alone, like KCl, did not apparently give rise to any toxic effect. Water consumption was increased by the administration of biphenyl, and the effect was appreciably enhanced by supplementing the diet with

NaHCO3 or KCl, in particular, among the salts. Water consumption was not enhanced with KHCO3 supplementation.

Enlargement of the left-side kidney, dilatation of the ureter, obstruction of part of the ureter and blood-containing urine were observed. Urinary calculi were not observable in the urinary bladder in any group.

The transitional epithelium in the obstructed ureter had seven to eight cell layers, in contrast with the transitional epithelium of the nonobstructed ureter, with three or four cell layers, and simple hyperplasia accompanied by a reduction of space in the ureteral lumen had taken place. Furthermore, slight cellular degeneration was occasionally seen in the transitional epithelium.

Physicochemical properties of the urine and urine crystals:
Precipitates were macroscopically observed in BP, BP + KHCO3, BP + KCl, BP + NaHCO3, CONTROL + KHCO3 and CONTROL + KCl, but not in the CONTROL. Urinary volumes were increased with the administration of KCl, KHCO3 and NaHCO3 (Table 2). Lamellar crystal clusters were observed only in BP + KHCO3. 4-HBPOSK was the major contituent of urine crystals as previously reported for urinary calculi (Ohnishi et al., 2000a).

Solubility of 4-HBPOSK in urine or plasma:
The solubility of 4-HBPOSK in urine was lower relative to that in plasma for each group (Table 3). The solubility of 4-HBPOSK in urine from CONTROL + KHCO3 and CONTROL + KCl was lower than the CONTROL, whereas no significant difference in the solubility of 4-HBPOSK in plasma was found among the three groups. It should be noted that the solubility of 4-HBPOSK in the urine from BP, BP + KHCO3 and BP + KCl, respectively (Table 4) was about 20 times higher than the concentration in plasma from BP, BP + KHCO3 and BP + KCl (Table 5).

Comparison of concentrations of the biphenyl ester conjugates in rat urine and plasma:
Among the biphenyl sulfate conjugates, 4-HBPOSK, 3-HBPOS and 3,4-DHBP-3-OS with the sulfooxy group at the three or four position accounted for more than 70% of the total metabolites for each group species. Moreover, the concentration of 4-HBPOSK, the main component of urine crystals and urinary calculi, was appreciably lower in urine from BP + KHCO3 and BP + KCl, with the addition of potassium to diets, than in urine from BP + KCl among all groups.

The presence of biphenyl metabolites, three isomers of HBPOS, four isomers of DHBPOS and three isomers of THBOPS, was identified in rat plasma. The distribution of the biphenyl sulfate conjugates for each group is roughly classified into three groups: the major component 4-HBPOSK: minor components 3-HBPOS, 3,4-DHBP-3-OS, and 3,4-DHBP-4-OS; and rare components consisting of the other metabolites of biphenyl sulfate conjugates. No significant differences were found in the concentrations of 4-HBPOSK among all the groups.

Biphenyl metabolite contents of the ureter:
The component fractions of all biphenyl sulfate conjugates in the obstructed ureter agreed approximately with those in urine crystals (Ohnishi et al., 2000a). In the hydronephrosis rat, the amounts of each of the biphenyl sulfate conjugates in the obstructed ureter contents were greater than those in the contents of the ureter without obstruction. For both hydronephrosis and normal rats, the amount of 4-HBPOSK was appreciably greater than the amounts of other metabolites of biphenyl sulfate conjugates.

5. Toxicity

Id 92-52-4

Date

References:
Ohnishi, M., Yajima, H., Takemura, T., Yamamoto, S., Matsushima, T., and Ishii, T. (2000a). Characterization of hydroxy-biphenyl-O-sulfates in urine and urine crystals induced by biphenyl and KHCO3 administration in rats. J Health Science 46:301-305.

Attached Document :

TABLE 1
Final Body Weights, Food and Water Consumption Values and Incidence of Hydronephrosis and Bleeding in Urine of Rats

Treatment	Final body weight[a] (g)	Consumption[a] Food (g/day)	Consumption[a] Water[c] (mL/day)	Incidence[b] Hydronephrosis	Incidence[b] Bleeding in urine
CONTROL	308 ± 19	14.1 ± 1.1	18 ± 3	0/5	0/5
BP	264 ± 14**	12.7 ± 1.0**	32 ± 3**	0/5	0/5
BP + $KHCO_3$	216 ± 18**	10.3 ± 1.0**	35 ± 4**	3/5	3/5
BP + KCl	269 ± 7**	13.0 ± 0.3*	42 ± 4**	0/5	0/5
BP + $NaHCO_3$	262 ± 5**	14.1 ± 0.4	52 ± 8**	0/5	0/5
CONTROL + $KHCO_3$	292 ± 16	14.1 ± 0.5	23 ± 3**	0/5	0/5
CONTROL + KCl	304 ± 13	14.6 ± 0.9	33 ± 2**	0/5	0/5

[a] Values are means ± SD of five rats.
[b] Data of five rats.
[c] Data of the last day.
* Significantly different from CONTROL at $p < 0.05$.
** Significantly different from CONTROL at $p < 0.01$.

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TABLE 2
Urinary pH, Concentrations of Potassium and Sodium Ions in Urine, and Urinary Volume

Treatment	Urine data pH	Potassium (mEq/day)	Sodium (mEq/day)	Volume (mL/day)
CONTROL	7.7 ± 0.1[a]	1.99 ± 0.56	1.04 ± 0.35	8 ± 3
BP	7.2 ± 0.1**	2.42 ± 0.63	1.20 ± 0.32	20 ± 4**
BP + $KHCO_3$	8.4 ± 0.3**	6.70 ± 0.85**	1.34 ± 0.14	28 ± 5**
BP + KCl	6.6 ± 0.1**	10.89 ± 1.10**	2.11 ± 0.14**	33 ± 2**
BP + $NaHCO_3$	8.8 ± 0.0**	2.73 ± 0.78	13.40 ± 3.71**	42 ± 4**
CONTROL + $KHCO_3$	8.8 ± 0.1**	7.26 ± 1.04**	1.28 ± 0.22	13 ± 2*
CONTROL + KCl	7.3 ± 0.1**	10.79 ± 1.01**	1.65 ± 0.27**	22 ± 1**

[a] Values are means ± SD of five rats.
* Significantly different from CONTROL at $p < 0.05$.
** Significantly different from CONTROL at $p < 0.01$.

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TABLE 3
Solubility of 4-HBPOSK in Urine or Plasma

Treatment	Added sample	Solubility of 4-HBPOSK Urine (μg/mL)	Solubility of 4-HBPOSK Plasma (μg/mL)
CONTROL	4-HBPOSK	370	1021
CONTROL + $KHCO_3$	4-HBPOSK	164	1072
CONTROL + KCl	4-HBPOSK	153	1400

Note. 4-HBPOSK was added to either urine or plasma collected from CONTROL, CONTROL + $KHCO_3$, and CONTROL + KCl to a concentration of 10 mg/mL. The suspension was filtered through a membrane filter, and the filtered solution was mixed with acetonitrile, which was diluted with water and used for LC–MS/MS analysis.

5. Toxicity

Id 92-52-4
Date

Attached Document :

TABLE 4
Concentration of Biphenyl Sulfate Conjugations in Urine Following Administration of Biphenyl or Biphenyl + Salt to Rat

Treatment	2-HBPOS	3-HBPOS	4-HBPOSK	4,4'-DHBPOS	2,5-DHBPOS	3,4-DHBP-3-OS	3,4-DHBP-4-OS	2,3-DHBPOS	3,4,4'-THBPOS
BP	60.24 ± 4.30[a] (4.81)[b]	327.78 ± 16.45 (26.19)	384.24 ± 12.09 (30.70)	41.09 ± 3.15 (3.28)	14.83 ± 1.66 (1.18)	287.99 ± 10.64 (23.01)	20.84 ± 1.28 (1.66)	40.04 ± 4.03 (3.20)	74.67 ± 6.99 (5.97)
BP + $KHCO_3$	42.40 ± 9.95* (5.26)	223.16 ± 49.00* (27.70)	169.56 ± 16.03* (21.04)	33.17 ± 5.67* (4.12)	9.84 ± 2.96* (1.22)	225.47 ± 40.65* (27.98)	15.62 ± 4.00* (1.94)	34.04 ± 9.09 (4.22)	52.50 ± 12.70* (6.52)
BP + KCl	44.68 ± 4.59* (5.72)	242.57 ± 19.81* (31.03)	107.09 ± 14.72* (13.70)	30.23 ± 4.21* (3.87)	9.47 ± 1.34* (1.21)	230.18 ± 20.32* (29.45)	14.45 ± 2.02* (1.85)	36.06 ± 4.09 (4.61)	66.92 ± 6.82 (8.56)
Biphenyl + $NaHCO_3$	33.89 ± 3.38* (4.02)	228.33 ± 24.45* (27.06)	288.67 ± 36.83* (34.22)	24.08 ± 3.06* (2.85)	7.72 ± 1.29* (0.92)	184.80 ± 13.08* (21.90)	9.20 ± 0.98* (1.09)	24.02 ± 1.16* (2.85)	42.94 ± 6.83* (5.09)

[a] Values are means ± SD (μg/mL) of five rats.
[b] Contents on group (%).
* Significantly different from BP at $p < 0.01$.

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TABLE 5
Concentration of Biphenyl Sulfate Conjugations in Plasma Following Administration of Biphenyl or Biphenyl + Salt to Rats

Treatment	2-HBPOS	3-HBPOS	4-HBPOSK	4,4'-DHBPOS	2,5-DHBPOS	3,4-DHBP-3-OS	3,4-DHBP-4-OS	2,3-DHBPOS	3,4,4'-THBPOS
BP	0.57 ± 0.28[a] (0.79)[c]	2.36 ± 0.96 (3.26)	59.68 ± 5.62 (82.53)	0.44 ± 0.13 (0.60)	ND[b]	4.05 ± 1.32 (5.60)	4.79 ± 0.96 (6.62)	0.14 ± 0.12 (0.19)	0.28 ± 0.14 (0.39)
BP + $KHCO_3$	0.92 ± 0.37* (1.12)	5.50 ± 1.50** (6.65)	58.55 ± 4.57 (70.80)	0.44 ± 0.20 (0.53)	ND	8.70 ± 2.47** (10.52)	6.96 ± 0.56** (8.42)	0.95 ± 0.44** (1.15)	0.68 ± 0.52* (0.82)
BP + KCl	0.51 ± 0.12 (0.68)	4.07 ± 0.46* (5.51)	56.80 ± 4.44 (76.78)	0.13 ± 0.10** (0.18)	ND	5.81 ± 0.31 (7.86)	5.89 ± 0.34** (7.96)	0.38 ± 0.08 (0.52)	0.38 ± 0.21 (0.52)
BP + $NaHCO_3$	0.46 ± 0.16 (0.67)	2.10 ± 0.58 (3.03)	56.74 ± 4.64 (81.79)	0.46 ± 0.15 (0.67)	ND	4.45 ± 0.66 (6.42)	4.77 ± 0.48 (6.88)	0.03 ± 0.06 (0.05)	0.35 ± 0.12 (0.50)

[a] Values are means ± SD (μg/mL) of five rats.
[b] ND, not detected.
[c] Contents on group (%).
* Significantly different from BP at $p < 0.05$.
** Significantly different from BP at $p < 0.01$.

Conclusion : Biphenyl and KHCO3 are significantly involved in the mechanism of formation of urine crystals composed primarily of 4-HBPOSK. Only in the case of 4-HBPOSK among the biphenyl sulfate conjugates did precipitation occur in the presence of potassium ions in aqueous solution at room temperature, regardless of pH value, whereas, in the presence of sodium ions, precipitation did not take place for any of the biphenyl sulfate conjugates. The low solubility of 4-HBPOSK in urine was confirmed by solubility in the presence of excess potassium ions in urine from CONTROL + KHCO3 and CONTROL + KCl rats.

The amount of water consumed by the CONTROL + KCl rats was considerably less than by the CONTROL + KHCO3 rats. This difference was also observed in the urinary volume collected the last day of the study. The solubility of 4-HBPOSK in urine from control rats showed that the concencentration of 4-HBPOSK in urine for BP + KCl was less than the limit of solubility, whereas the concentration of 4-HBPOSK in urine for BP + KHCO3 was close to the limit of solubility.

Urine crystals with urinary calculi were not observed in this study. It is believed to be due to the shorter duration in this study than in the 104 week study where urinary calculi were observed.

Reliability : (2) valid with restrictions
2e Meets generally accepted scientific standards, well documented and acceptable for assessment

01.03.2005 (29)

Type : Chronic
Species : rat
Sex : male/female
Strain : Fischer 344/DuCrj
Route of admin. : oral feed
Exposure period : 105 weeks
Frequency of treatm. : daily
Post exposure period :
Doses : 4500 ppm
Control group : yes, concurrent no treatment
Method : other: part of OECD 453 guideline
Year : 1981

GLP : yes
Test substance :

Method : Study was conducted on calculi found in the urinary bladder of male and female rats fed 4500 ppm biphenyl for 105 weeks (Umeda et al., 2002).

Calculi were collected from the urinary bladder of rats at necropsy. They were immediately removed from the urine and dried. Both single and multiple calculi were observed in males and females.

The calculi were crushed and a portion was dissolved in acetonitrile (1:1, v/v). This solution, appropriately diluted with distilled water, was used for analyses of the biphenyl metabolite content and ion content by HPLC and ion chromatography (IC). Furthermore, a portion of the powdered calculus preparation was dissolved in a mixture of nitric acid and perchloric acid at 150C. This solution, appropriately diluted with distilled water, was used for analysis of the inorganic elements in the calculi by inductively coupled plasma spectroscopy (ICP).

Structural analysis of the calculi was conducted using micro Fourier transform infrared spectroscopy (mFT-IR) spectra analysis. In addition, the whole exposed surface of the cut calculus was coated with acrylic resin for elemental analysis by electron probe microanalyzer (EPMA).

Test substance : Biphenyl, CAS# 92-52-4. Purity greater than 98% was obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan.

Result : Sex differences in calculus characteristics were apparent in terms of the surface-staining colors and the shapes of the calculi. The surface-staining colors of the urinary calculi in male rats varied over a wide color range including white, yellow, brown, gray or black, whereas the colors of calculi from female rats were either white or yellow. The shapes of urinary calculi in male rats varied, including spheroid, triangular pyramidal or cubical whereas the majority of the calculi in females were spheroid.

The average contents of biphenyl metabolites in the calculi analyzed by HPLC are shown in Table 1. Males had a much higher percentage of 4-hydroxy-biphenyl-O-sulfate (4-HBPOSK) whereas females had a much higher percentage of 4-hydroxy-biphenyl (4-HBP).

There was no apparent difference in potassium, sulfur, calcium and phosphorus levels in calculi from male or female rats. However, calculi from female rats had 23 times higher sulfate levels than did calculi from male rats and several other differences were identified (Table 2).

In a cross-section, the calculi of male rats was found to have a multilayer structure. The thickness of each of the layers was about 250 um and open holes between the layers were observed in places. The 4-HBPOSK content tended to increase going from the outer layer to the center of the calculi in male rats.

However, in a cross-section, the calculi of female rats was found to lack the multilayer structure. Open holes were observed in places. Needle-shaped crystals were found in the holes. The length of the needles was about 150-200 um. Based on IR analysis the needle-shaped crystals were consistent with 4-HBP. The area around the open holes was consistent with KHSO4 while the outer area of the calculus was consistent with calcium phosphate.

Attached Document :

Table 1. Contents of the Biphenyl Metabolites in Urinary Calculi in Male and Female Rats

	biphenyl metabolite content (%)[a]		retention time (min)
	male[b]	female[c]	
sulfate			
4-HBPOSK	65.3 ± 9.80[d]	3.40 ± 0.90	1.7
4,4′-DHBPOS	1.08 ± 0.49	ND[e]	1.4
hydroxide			
4-HBP	1.34 ± 3.04	44.4 ± 5.10	5.5
4,4′-DHBP	ND	0.59 ± 0.30	2.6
2-HBP	ND	0.10 ± 0.08	6.0
3,4-DHBP	ND	0.04 ± 0.05	3.6

[a] With respect to the reliability of the results of the analyses, the correlation coefficient and the coefficient of variation in first-order regression analyses for the calibration curve of each compound were more than 0.999 and less than 5%, respectively, for 50 samples in the concentration range of 0.5−10 μg/mL. [b] $n = 34$. [c] $n = 4$. [d] Mean ± SD. [e] ND, not detected.

Attached Document : Table 2

Table 3. Comparison of the Physicochemical Characteristics of Urinary Calculi in Male and Female Rats

	male	female	methods[a]
shape	spheroid, triangular pyramidal, cubical	spheroid	
size[b]	0.3−1.0 cm	0.3−1.0 cm	
color	white, yellow, brown, gray, black	white, yellow	
main constituent	4-HBPOSK	4-HBP	HPLC
		sulfate	IC
principal elements	S, K, P, Ca	S, K, P, Ca	ICP
structure	multilayer composed of inside alternating layers (S−K layer and P−Ca layer) and an outside thick layer	stone-like lump with the center hole, where the needle crystal exists, and outside thin layer	microscopy, EPMA
components constituting a fine structure	inner layer, 4-HBPOSK	open hole needle, 4-HBP	mFT-IR
	outer layer, $Ca_3(PO_4)_2$	open hole surrounding area, $KHSO_4$	
distribution of principal elements	inside alternating layers, S−K and P−Ca layers	inside layer, S−K	EPMA
	outside thick layer, P−Ca	outside thin layer, P−Ca	

[a] Sample number of analysis: 34 males and 4 females for HPLC and 3 males and 2 females for IC and ICP. [b] Minimum to maximum size.

Remark : According to the authors, they identified approximately 95% of the total contents in the calculi in males whereas, in females, only 80% was characterized.

According to the authors, the pH of urine from high dose male rats sacrificed at the end of the 104 week study ranged from 7.5 - 8.5 whereas the pH of urine from high dose female rats ranged from 6.5 - 8.0. The control male and female urine pH values were comparable and in both cases were in the range of 6.5 - 8.0. The more acidic nature of the urine in female rats is thought to be significantly involved in the hydrolysis of 4-HBPOSK, and the basic environment of the urine in males is thought to govern the formation of calculi.

The authors also suspect that a specific hydrolytic enzyme involved in sulfate conjugation, aryl-sulfatase, present in the kidney has higher activity in female rats than in males.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

01.03.2005 (30)

Type : Sub-chronic
Species : mouse
Sex : male/female
Strain : other: BDF1
Route of admin. : oral feed
Exposure period : 13 weeks

Frequency of treatm.	:	
Post exposure period	:	
Doses	:	500, 2,000, 4,000, 8,000, 10,000 or 16,000 ppm
Control group	:	yes, concurrent no treatment
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method :

Male and female Crj: BDF1 mice (SPF), 4 weeks old were obtained from Charles River Japan. Animals were acclimated for 2 weeks and divided by stratified randomization into 7 weight-matched groups, each consisting of 10 mice of each sex. A diet containing biphenyl at 0 (control), 500, 2,000, 4,000, 8,000, 10,000 or 16,000 ppm (w/w) was prepared by mixing the chemical with gamma-irradiation sterilized CRF-1 powdered diet (Oriental Yeast Company, Toyko, Japan). The diet was stored at 4C until use. Groups of 10 male and 10 female mice had free access to UV-irradiated, sterilized and filtered water and either the control diet or each of 6 biphenyl-containing diets of different concentrations throughout the 13-week period, starting at the age of 6 weeks. In order to avoid possible taste aversion in the mice fed high doses of biphenyl, the dose levels were increased weekly in a stepwise fashion. The mice fed biphenyl at 8,000 or 10,000 ppm were given the 4,000 ppm containing diet for the first week and then 8,000 or 10,000 ppm for the remaining 12 weeks, respectively. The mice fed the highest dose of biphenyl were given the 4,000 ppm diet for the first week, followed by the 8,000 ppm diet for the second week and finally the 16,000 ppm diet for the remaining 11 weeks. The biphenyl concentrations in the diets were confirmed by gas chromatography, and found to be constant, within a range of 99.2% to 112% of the designated target concentration.

All animals were observed daily for clinical signs and mortality. Body weight and feed consumption were measured weekly. Organs were removed, weighed and examined. The tissues for light microscopic examination were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections 5um thick were prepared and stained with hematoxylin and eosin (H&E stain). The tissues from 2 female mice, one fed the control and one the 16,000 ppm diet, were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed in 2% osmium tetroxide, and then embedded in epon-resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Hitachi, H-7000, Tokyo, Japan).

Body weight, feed consumption and organ weights were analyzed by Dunnett's test.

Result :

All mice except a 16,000 ppm-fed female survived to the end of the 13-week feeding period. Final body weights of mice of both sexes fed 8,000, 10,000 and 16,000 ppm were significantly lower than their respective controls (for males: 83.3%, 64.9% and 75.1%, for females: 93.7%, 91.6% and 85.5%, respectively). Absolute liver weights were significantly higher in the female 8,000 and 16,000 ppm groups than those in the female control group.

Light microscopically, the centrilobular hepatocytes of all the surviving female mice fed 16,000 ppm were greater than the normal hepatocytes of the female control group. The cytoplasm of the enlarged hepatocytes was filled with numerous eosinophilic fine granules.

Electron-microscopically, many spherical organelles were observed in the cytoplasm of the enlarged hepatocytes of the 16,000 ppm-fed female mice. Those organelles were about 0.7 um in diameter, had single membrane and were homogeneous with low density. The eosinophilic fine granules in the centrilobular hepatocytes were identified as peroxisomes on the basis

of electron microscopy. On the other hand, the eosinophilic fine granules, the lesion suspected in peroxisome proliferation, were not recognized by use of light microscopy in the liver of biphenyl-fed male mice.

Remark : For a 20 gram mouse ingesting 5 grams/day, dose levels of 500, 2000, 4000, 8000, 10,000 and 16,000 ppm correspond to 125, 500, 1000, 2000, 2500 and 4000 mg/kg/day, respectively.

In personal communication with Dr Umeda, the absolute and relative liver weights for the various dose levels in the 13 week study were as follows:

Table 2. Absolute and relative liver weights of both male and female BDF1 mice in the 13-week study

Group (ppm)	Control	500	2000	4000	8000	10000	16000
Male							
No. of animals[a]	10	10	10	10	9	10	10
Body weight[b]	29.4 ± 1.6	28.7 ± 1.5	28.7 ± 2.3	26.9 ± 0.6	24.4 ± 1.9	25.1 ± 1.5	21.9 ± 1.2
Absolute (g)	1.044 ± 0.053	1.044 ± 0.052	1.066 ± 0.082	1.099 ± 0.042	1.090 ± 0.094	1.084 ± 0.052	1.077 ± 0.044
Relative (%)	3.556 ± 0.230	3.638 ± 0.092	3.709 ± 0.076	4.092 ± 0.127	4.468 ± 0.113	4.324 ± 0.171	4.931 ± 0.113
Female							
No. of animals[a]	10	10	10	10	10	10	9
Body weight[b]	21.4 ± 1.1	21.7 ± 1.5	21.5 ± 1.3	20.7 ± 0.8	20.3 ± 0.7	19.4 ± 1.0	18.6 ± 0.5
Absolute (g)	0.854 ± 0.038	0.883 ± 0.063	0.874 ± 0.051	0.899 ± 0.055	0.996 ± 0.050	0.897 ± 0.074	1.009 ± 0.064
Relative (%)	3.989 ± 0.194	4.065 ± 0.201	4.063 ± 0.192	4.354 ± 0.205	4.917 ± 0.145	4.631 ± 0.194	5.411 ± 0.270

Mean±S.D.

a: The values indicate number of animals surviving to the end of 13-week administration period

b: The data indicate the mean body weights in survival animals which fasted overnight after at the end of 13-week administration period

The four highest doses in this study were greater than the highest dose used by Sunouchi et al., 1999. At the highest dose, 800 mg/kg, that Sunouchi et al., 1999 used, they reported no effect on liver weight. In this study, there was essentially no effect on liver weight in female mice at 500 mg/kg (2000 ppm) and a 9% increase at 1000 mg/kg (4000 ppm) after 13 weeks of administration. Thus it is not surprising that Sunouchi et al., 1999 did not observe a liver weight increase.

The concentrations used in this study were much higher than the hghest concentration used in the two year bioassay, 6000 ppm.

Conclusion : The 13-week oral administration of biphenyl at 16,000 ppm through diet feeding to female BDF1 mice was found to induce peroxisome proliferation. No such effect was observed in male mice fed the same doses.

Reliability : (2) valid with restrictions
2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

28.02.2005 (31)

Type : Sub-acute
Species : mouse
Sex : male/female
Strain : other:BDF1
Route of admin. :
Exposure period : 3 days
Frequency of treatm. : daily
Post exposure period :
Doses : 1.3, 2.6 and 5.2 mmol/kg (corresponds to 200, 400 and 800 mg/kg)
Control group : no data specified
Remark : This paper suggests a sex difference exists with females showing evidence of peroxysome proliferation at a molecular level.

The response observed at 5.2 mmol/kg suggests biphenyl is a relatively weak peroxysome proliferator when compared with the response observed for clofibrate. Clofibrate itself is considered a moderate peroxysome proliferator.

Attached document :

21C-03-3

Effects of Diphenyl on Hepatic Peroxysomal Enzyme and Drug-Metabolizing Enzyme Activities in BDF1 Mice.

Momoko SUNOUCHI, Atsuko MIYAJIMA, Shogo OZAWA and Yasuo OHNO Div. Pharmacol., Natl. Inst. Health Sci., Tokyo 158-8501, Japan

The effects of diphenyl on hepatic peroxisomal enzyme and drug-metabolizing enzyme activities were studied in BDF1 mice. In female BDF1 mice, oral administration of diphenyl (1.3, 2.6 and 5.2 mmol/kg) for 3 days significantly increased KCN-insensitive palmitoyl CoA (PCoA) oxidation in liver homogenates (up to 1.9-fold) and lauric acid (LA) 12-hydroxylation in liver microsomes (up to 3.8-fold). This increase of LA 12-hydroxylation was paralleled by enhanced P450 protein level as determined by immunochemical analysis using anti-rat CYP4A antibody. However, these increases of PCoA oxidation and LA 12-hydroxylation by diphenyl were lower than those (8.6-fold and 16-fold) by clofibrate (2.6 mmol/kg). In both sexes, diphenyl (5.2 mmol/kg) increased pentoxyresorufin O-depentylation (PROD) (1.8-fold in female; 2.3-fold in male) and P450 protein level as determined by immunochemical analysis using anti-rat CYP2B antibody. Diphenyl caused no change of the relative liver weight. On the other hand, in the male mice PCoA oxidation and LA 12-hydroxylation were not increased after the administration of diphenyl. These results indicate that diphenyl exhibits characteristics of a CF-type hepatic enzyme inducer in female BDF1 mice and of a PB-type in female and male BDF1 mice.

Reliability : (4) not assignable
4a: Abstract

10.06.2005 (32)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium
Test concentration	:	0 to 100 micrograms per plate
Cycotoxic concentr.	:	100 micrograms/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: NTP
Year	:	
GLP	:	no data

5. Toxicity

Id 92-52-4
Date

Test substance :

Method : As each strain of Salmonella typhimurium is genetically different, using several strains in a test increases the opportunity of detecting a mutagenic chemical. All strains of Salmonella typhimurium used for mutagenicity testing carry a defective (mutant) gene that prevents them from synthesizing the essential amino acid histidine. Mutations leading to the ability to synthesize histidine are called "back" or "reverse" mutations and the process is referred to as "reversion."

Some test protocols utilize extracts of Aroclor-induced rat or hamster liver enzymes (S9) to promote metabolic conversion of the test chemical. This is necessary since the Salmonella bacterium does not have mammalian metabolic capabilities.

In the Salmonella assay, a test tube containing a suspension of one strain of Salmonella typhimurium plus S9 mix or plain buffer without S9, is incubated for 20 minutes at 37º C with the test chemical. Control cultures, with all the same ingredients except the test chemical, are also identically incubated. In addition, positive controls with a known potent mutagen, are prepared. After 20 minutes, agar is added to the cultures and the contents of the tubes are thoroughly mixed and poured onto the surface of Petri dishes containing standard bacterial culture medium. The plates are incubated, and bacterial colonies that do not require an excess of supplemental histidine appear and grow. These colonies are comprised of Salmonella that have undergone reverse mutation to restore function of the histidine-manufacturing gene. The number of colonies is counted after 2 days.

Several doses (at least 5) of each test chemical and multiple strains of Salmonella typhimurium are used in each experiment. In addition, cultures are set up with and without added S9 liver enzymes at 10% concentration in these studies.

The pattern and the strength of the mutant response are taken into account in determining the mutagenicity of a chemical. All observed responses are verified in repeat tests. If no increase in mutant colonies is seen after testing several strains under several different culture conditions, the test chemical is considered to be nonmutagenic in the Salmonella test.

No additional information provided.

Reference
Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000 Nov 20;455(1-2):29-60

Result : Data found on NTP public database at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

Study ID 512660
Solvent DMSO
Preincubation

Strain: TA100

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	173	24.3	116	3.8	146	2.8	157	7.8	156	10.9	155	7.5
1	173	14.8	110	6.9	169	13	182	0.7	165	6.5	164	6.6
3.3	176	15.3	103	3.4	158	7.9	160	7.8	153	3.6	189	18.3
10	146	23.2	101	10	151	3.3	164	2.8	164	3.4	118	45

33	135	51.7	85	6.2	152	4.9	165	2.2	151	12.5	175	13.6
100	90	42.5	67	9	154	9.9	163	15.1	160	6.7	161	12.7
P Con	447	32.4	375	19.9	297	5.7	357	14.9	641	81	587	49.8

Strain: TA1535

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	12	1.2	4	0.9	10	0.3	9	0.7	13	0.6	6	0.9
1	11	1.2	7	1.8	6	1	6	0.3	11	0.3	4	0.9
3.3	9	1.5	6	1.5	6	1.3	6	1	9	2	4	0
10	5	1.5	4	1.9	8	1	6	0.9	7	1.5	4	0.7
33	10	2.3	5	0.9	6	2.8	6	0.7	7	1.5	6	1.7
100	11	1.9	5	1.2	9	2.1	7	0.6	9	0.3	9	2.4
P Con	269	9.1	333	25.9	29	4.8	18	0.6	42	7.2	29	2.7

Strain: TA1537

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	7	1	6	0.9	9	0.6	9	1.2	9	1.2	9	0.6
1	5	1.5	6	1.5	6	1.5	12	3	7	1.5	8	0.7
3.3	5	0.9	5	0.9	5	0.9	11	3.2	8	1.2	8	0.6
10	7	0.6	6	1.2	8	1.2	8	0.3	5	0.6	8	1.5
33	3	0.6	6	1.7	7	1.5	7	0.3	8	2.3	8	0.3
100	4	1.5	6	1.5	7	1.2	7	0.3	6	1.2	6	0.6
P Con	297	31.7	122	5.8	21	3.5	36	11.6	75	16.5	64	3.9

Strain: TA98

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	15	1.5	14	0.6	19	1.2	19	1.2	19	3.7	19	6.3
1	15	3	15	1.5	19	3.1	23	2.3	19	1.9	28	4.6
3.3	13	1.5	11	1.3	22	5.4	20	1.2	20	1.7	31	5.9
10	11	3.5	12	1.2	18	2.1	15	1.5	18	1.7	29	6.4
33	8	1.3	13	3.8	20	1.7	21	1.8	16	2.7	26	5
100	7	0.6	13	3.5	22	0.9	17	2.6	19	3.5	23	2.2
P Con	283	14.2	266	4.5	189	16	333	22.4	610	93.7	718	101

Study ID 773612
Solvent DMSO
Preincubation

Strain: TA100

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	102	3.2	73	4.4	91	3.2	86	2.5	88	4.1	68	4.1
1	109	6.9	78	3.7	97	5	84	4.6	82	3.8	73	5.5
3	92	7.4	76	2.9	93	4.7	79	1	85	0.9	63	3.5
10	103	7.8	81	5	94	9.9	73	0.3	84	10.5	62	3.2
33	76	2.9	59	3.5	87	8.6	74	7	89	0.7	74	3.9
100	87s	0.9	58	6.4	93	5.3	60	0.3	86	4	61	4.4
P con	1955	92	1864	48.3	493	57	1432	62.5	3349	123	2649	267

Strain: TA1535

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	9	3	18	1.2	7	1.2	10	0.9	7	2.2	12	0.9
1	12	0	18	2.1	12	3.2	9	2	11	0.3	11	0.3

3	10	1.2	18	2	8	1.5	10	1.2	13	1.5	5	0.9
10	8	2.6	23	4	8	0.3	9	1.2	9	2	11	2
33	5	1.9	17	2.6	11	1	8	2.2	10	2.3	8	2
100	179	0	14s	0.9	5s	2	9	1.8	6s	1.3	8s	1
P con	857	19	887	142	55	4.7	81	3.3	295	11.1	123	8.8

Strain: TA1537

Dose	No MA		No MA		RLI		RLI		HLI		HLI	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	6	1.2	3	0.9	5	0.9	9	3.2	6	0.3	7	1.2
1	7	1.2	5	0.9	7	0.7	7	0.3	6	1.5	7	1.9
3	5	0.9	9	1.5	9	1.7	5	1.5	7	0.9	4	0.9
10	7	0.7	7	2.5	7	2.8	6	1.5	5	0.3	6	1.7
33	4	1.5	3	1.7	7	1.5	7	2.8	5	0.3	7	1.5
100	3s	0.7	3s	1	6	2	5	1.2	6	0	4	1.5
P con	503	93	192	65.3	64	16	55	2.6	285	9	255	5.8

Strain: TA98

Dose	No MA		No MA		RLI		RLI		HLI		HLI	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	16	1.8	27	5.2	16	0.6	21	3.9	25	5	25	2.6
1	15	1	22	3.3	19	1.9	22	2.4	21	0.3	21	2.7
3	17	0.6	22	3.8	19	2.5	24	1.2	24	1.2	18	2.7
10	19	3.4	20	2.6	16	1.5	22	4	19	1.3	19	2.1
33	12	2.6	11	1.2	19	1	20	2.3	19	4.7	23	2.5
100	10s	0.9	12	1.2	20	1.2	18	1.2	20	2.3	19	2.1
P con	1207	34	1564	29.2	384	48	1322	35.8	2525	251	2748	127

S = Slight Toxicity
MA = Metabolic Activation
RLI = Rat Liver, Induced
HLI = Hamster Liver, Induce

Remark : This result is also supported by the following reports of negative Ames tests on Biphenyl:

Bos RP, Theuws JLG, Jongeneelen FJ, Henderson PT (1988) Mutagenicity of bi-, tri- and tetra-cyclic aromatic hydrocarbons in the "taped-plate assay" and in the conventional Salmonella mutagenicity assay. Mutation research, 204:203-206.

Brams A, Buchet JP, Crutzen-Fayt MC, de Meester C, Lauwerys R, Leonard A (1987) A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure). Toxicology letters, 38:123-133.

Fujita H, Kojima A, Sasaki M, Hiraga K (1985) Mutagenicity test of antioxidants and fungicides with Salmonella typhimurium TA97a, TA102. Kenkyu Nenpo-Tokyo-toritsu Eisei Kenkyusho, 36:413-417.

Glatt H, Anklam E, Robertson LW (1992) Biphenyl and fluorinated derivatives: liver enzyme-mediated mutagenicity detected in Salmonella typhimurium and Chinese hamster V79 cells. Mutation research, 281:151-156

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983) Salmonella mutagenicity test results for 250 chemicals. Environmental mutagenesis, 5 (Suppl. 1):3-142.

Ishidate M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M,

Matsuoka A (1984) Primary mutagenicity screening of food additives currently used in Japan. Food and chemical toxicology, 22:623-636.

Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T (1980) Cooperative programme on short-term assays for carcinogenicity in Japan. In: Montesano R, Bartsch H, Tomatis L, eds. Molecular and cellular aspects of carcinogen screening tests. Lyon, International Agency for Research on Cancer, pp. 323-330 (IARC Scientific Publications No. 27).

NTP (1980) Annual plan for fiscal year 1981. Research Triangle Park, NC, US Department of Health and Human Services, National Toxicology Program, p. 32.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. Environmental mutagenesis, 3:11-32.

Purchase IFH, Longstaff E, Ashby J, Styles JA, Anderson D, Lefevre PA, Westwood FR (1978) An evaluation of 6 short-term tests for detecting organic chemical carcinogens. British journal of cancer, 37:873-959.

Bronzetti G, Esposito A, Pagano G, Quinto I (1981) A comparative study on the toxicity and mutagenicity of biphenyl (BP) and diphenyl ether (DPE) in sea urchin, S. typhimurium and S. cerevisiae. Mutation research, 85:233.

Cline JC, McMahon RE (1977) Detection of chemical mutagens. Use of concentration gradient plates in a high capacity screen. Research communications in chemical pathology and pharmacology, 16:523-533.

Pagano G, Esposito A, Giordano GG, Vamvakinos E, Quinto I, Bronzetti G, Bauer C, Corsi C, Nieri R, Ciajolo A (1983) Genotoxicity and teratogenicity of diphenyl and diphenyl ether: a study of sea urchins, yeast, and Salmonella typhimurium. Teratogenesis, carcinogenesis, and mutagenesis, 3:377-393.

Pagano G, Cipollaro M, Corsale G, Della Morte R, Esposito A, Giordano GG, Micallo G, Quinto I, Staiano N (1988) Comparative toxicity of diphenyl, diphenyl ester, and some of their hydroxy derivatives. Médecine Biologie Environnement, 16:291-29

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance :
Biphenyl, CASNO 92-52-
Conclusion : Material was non-mutagenic in the presence or absence of a standard liver metabolic activating system.
Reliability : (1) valid without restriction
High quality study with multiple-species activating system and independent confirmation
Flag : Critical study for SIDS endpoint
08.02.2005 (33)

Type : Chromosomal aberration test
System of testing : Chinese hamster lung
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result :
Method :
Year : 1985
GLP : no data
Test substance :

Method : For the probe study, dose levels of 0, 0.075, 0.1 and 0.125 mg/ml biphenyl were used without metabolic activation. The solvent used was DMSO. Treatment times were 24 and 48 hours.

For the definitive study, dose levels of 0, 0.01, 0.015, 0.02 mg/ml were used with and without metabolic activation. The solvent used was DMSO. No information on treatment time was provided in the Table of the report (see attachment).

No additional information was provided.

The following method description is from:
Mutagenicity Test Data of Existing Chemical Substances (1996) by Japan Chemical Industry Ecology-Toxicology & Information Center (JETOC), Japan

The methods supplied in this document reflect the methods in use in 1985 of the chromosomal aberration test in cultured mammalian cells, specifically Chinese Hamster lung cells.

Chinese hamster lung (CHL) cell line isolated by Dr Ishidate, Jr et al., of the National Institute of Hygienic Sciences, Tokyo, Japan was used.

Saline was used for water soluble materials and dimethylsulfoxide (DMSO) was used for materials insoluble in water but soluble in DMSO. The test substance solution for DMSO was 0.025 ml with 5.0 ml medium.

For this test, S9 derived from the liver of Sprague-Dawley rats induced with sodium phenobarbital and 5,6-benzoflavone was used. The final concentration of the S9 fraction in the final treatment medium was 5%.

Cell Growth Inhibition study
The cell growth inhibition study is used to check for cytotoxicity.

Without Metabolic Activation (24 and 48 hour treatments)
2 x 10(4) cells were seeded with 5 ml of culture medium in a Petri dish and cultured for three days. Then the test substance solution was added to a dish of culture medium. After 24 or 48 hours treatment, rates of cell growth inhibition were determined.

With Metabolic Activation (6 hours treatment)
2 x 10(4) cells were seeded with 5 ml of culture medium in a Petri dish and cultured for three days. Cells were simultaneously treated with S9Mix and the test substance solution for 6 hours. Then the test substance mixture was changed to fresh culture medium. After 18 hours further culture, rates of cell growth inhibition were determined.

Negative Control
The cells treated with solvent alone served as negative control.

Determination of Cell Growth Inhibition Rate
The cells were washed with physiological saline. The cells were fixed with ethanol and stained with 0.1% crystal violet. The cell growth index was calculated with the negative control being 100% and the Petri dish without cells being 0%.

Chromosomal Aberration Test
The test was carried out at several (3 to 5) different concentrations of test substance selected from the result of cell growth inhibition study. Two culture vessels were used for each concentration of test substance.

Without metabolic inhibition (24 and 48 hours treatment)
2 x 10(4) cells were seeded with 5 ml of culture medium in a Petri dish and cultured for three days. Then the test substance solution was added to a dish of culture medium. After 24 or 48 hours treatment, chromosome slide preparations were made.

With metabolic inhibition (6 hours treatment)
2 x 10(4) cells were seeded with 5 ml of culture medium in a Petri dish and cultured for three days. Cells were simultaneously treated with S9Mix and the test substance solution for 6 hours. Then the test substance mixture was changed to fresh culture medium. After 18 hours further culture, chromosome slide preparations were made.

Positive control chemicals and their final concentrations were as follows
without metabolic activation
Mitomycin C 0.04 ug/ml

with metabolic activation
Benzo[a]pyrene 10 ug/ml
Cyclophosphamide 10 ug/ml

Slide preparation
Cells were treated with 0.2 ug/ml colcemid for 2 hours and after treatment with trypsin incubated with 75mM hypotonic KCl for 20 minutes at 37C. The cells were fixed with acetic acid-ethanol (1:3) and spread onto clean glass slides. After air-drying, each slide was stained with Giemsa solution (2.5% at pH 6.8) for 12 minutes.

Chromosome Observation
The number of cells with chromosomal aberrations from 100 metaphases were counted per each culture bottle. The types of aberrations were classified into 6 groups: chromatid and chromosome gaps, chromatid breaks, chromatid exchanges, chromosome breaks, chromosome exchanges, and others (fragmentations). The incidence of polyploid cells in the 100 metaphases was recorded.

Evaluation
Frequencies of structural aberrations and of polyploidy of less than 5% were considered negative, from 5-<10% was considered equivocal and 10% or more was considered positive.

Test substance : Purity of the test substance was not defined. A JETOC study conducted in 1989 used 99.5% pure biphenyl

Result : Biphenyl was positive only in the presence of S9 mix (Table 2).

Attached document : Table 1

Table 2. Chromosome aberration tests in cultured Chinese hamster cells (Direct method) (1982)

Compound	Solvent	Dose (mg/ml)	Treatment time (h)	Polyploid (%)	Frequency (%) of aberrant cells† ctg	ctb	cte	csb	cse	Total	Judge
Biphenyl	DMSO	0	24	0.0	1.0	1.0	0.0	0.0	0.0	2.0	
		0.075	24	0.0	1.0	0.0	0.0	0.0	0.0	1.0	−
		0.1	24	1.0	0.0	0.0	0.0	0.0	0.0	0.0	−
		0.125	24	1.0	0.0	4.0	0.0	0.0	0.0	4.0	−
		0	48	0.0	0.0	0.0	1.0	0.0	0.0	1.0	
		0.075	48	0.0	1.0	0.0	0.0	0.0	0.0	1.0	−
		0.1	48	2.0	1.0	0.0	0.0	0.0	1.0	2.0	−
		0.125	48	2.0	2.0	0.0	0.0	0.0	0.0	2.0	−

Attached document : Table 2

Table 5. (continued)

Compound	Solvent	S 9	Dose (mg/ml)	Polyploid (%)	Frequency (%) of aberrant cells†						Judge
					ctg	ctb	cte	csb	cse	total	
Biphenyl	DMSO	−	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
		−	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	−
		−	0.015	0.0	1.0	0.0	0.0	0.0	0.0	1.0	−
		−	0.02	1.0	0.0	0.0	0.0	0.0	0.0	0.0	−
		+	0	1.0	1.0	0.0	1.0	0.0	0.0	2.0	
		+	0.01	4.0	2.0	0.0	3.0	0.0	0.0	5.0	±
		+	0.015	1.0	0.0	10.0	31.0	0.0	0.0	35.0	+
		+	0.02	1.0	4.0	35.0	28.0	0.0	0.0	51.0	+

Remark : Original document is written primarily in Japanese. Only abstract and tables are in English.

Reliability : (2) valid with restrictions
2D Meets national standard methods with acceptable restrictions.

02.05.2005 (34)

Type : Cytogenetic assay
System of testing : Syrian Hamster cell line: DON
Test concentration : 0.1, 0.2, 0.5 or 1.0 mM
Cycotoxic concentr. : 1.0 mM showed mitotic inhibition
Metabolic activation : without
Result : negative
Method :
Year :
GLP : no
Test substance :

Method : The purpose of this study was to compare chromosome aberrations and sister chromatid exchange frequency for several chemicals under identical culture conditions. In this study a pseudodiploid Chinese hamster cell line (Don) was exposed using three to five concentrations of the test materials. In the case of Biphenyl, concentrations of 0.1, 0.2, 0.5 or 1.0 mM test material were incubated with cells in Eagle's MEM with 10% FCS and 1 microgram per ml BudR for 26 hours (2 rounds of cell division) at 37° C in compete darkness. Colchicine (0.25 mcgm/ml) was added for the last two hours of incubation. Cells were collected using a rubber policeman and air-dried slides were prepared following hypotonic treatment for 20 minutes and fixation in ice-cold methanol:acetic acid (3:1). Slides of chromosome aberration examination were prepared by conventional Giemsa staining. Separate slides received special stains for determining SCEs.

Slides were scored by examining 100 metaphases for each concentration and the frequency of aberrations, excluding gaps, was estimated by the number of breaks per cell. A ring, a dicentric and a chromatid exchange were each scored as two breaks, a tricentric as four breaks, and an acentric or isochromatid break were scored as one break.

SCE's were scored by a different investigator and 20-50 intact metaphases per concentration in which all metaphases had a "harlequinized" appearance without gross chromosome aberration.

The criterion for a positive result was set at a dosage-related increase in aberrations of at least twice that of controls. Positive substances were also run as part of the study

Result : The high concentration (1.0 mM) produced some toxicity as evidenced by an inhibition of mitotic activity.

Results of the scoring for "breaks" and "exchanges" are:

Conc (mM)	breaks/cell	SCE/cell
0.0	0.06	8.17
0.1	0.10	10.37
0.3	0.12	9.06

0.5	0.03	10.33
1.0	0.08	13.12
pos cont*	>7.77	18.44

Positive control was N-n-butyl-N-nitrosourethane at 1 mM for chromosome aberrations and at 0.1 mM for SCE

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance : Biphenyl, CASNO 92-52-4
Conclusion : Biphenyl did not produce an increase in chromosome aberrations or SCEs under these conditions, negative and positive controls gave the expected results
Reliability : (4) not assignable
4e: Documentation insufficient for assessment.
Published report is assigned a reliability of 4. Despite differences from the current OECD 473 guidance, the information is considered reliable, as results of a large range of compounds were available providing validation of the methodology. Differences from OECD 473 were that there was no metabolic activation system used, cytotoxicity was not determined and it may have been possible to use a higher concentration (10 mM is the highest concentration recommended by the OECD 473 guideline) and the number of metaphases examined was half that recommended by the current guideline.

02.05.2005 (35)

Type : Mammalian cell gene mutation assay
System of testing : Chinese Hamster V79 cells
Test concentration : 0, 10, 25, 50 and 100 ug/mL.
Cycotoxic concentr. :
Metabolic activation : with and without
Result :
Method : other: Essentially follows OECD 476
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Chinese hamster V79 cells were maintained in Dulbecco's modified minimum essential medium supplemented with fetal calf serum (5%), penicillin (100 units/ml) and streptomycin (100 ug/ml). Cells were grown at 37C in a humidified atmosphere containing 5% CO2. A total of 1.5 x 10(6) cells and 30 ml medium were added to each 15-cm petri dish. After 18 hr, the medium was replaced by 18 ml S9 mix or PBS-HEPES, and the test compound (dissolved in 60 ul dimethyl sulfoxide) was added. S9 mix or buffer and the test compound were removed 2 hours later. Medium (30 ml) was added to the cultures after washing with PBS-HEPES. After an expression period of 6 days with one subculture, cells were replated at a density of 10(6)/15-cm Petri dish in medium containing 6-thioguanine (7 ug/ml) for the selection of mutants (6 dishes) or, at a density of 100 cells/6-cm Petri dish in medium without 6-thioguanine, for the determination of the cloning efficiency (3 dishes). The cultures were fixed and stained, and the colonies were counted after about 7 days (cloning efficiency) or 10 days (mutants). Mutant frequencies were calculated.

Cytotoxicity in the mutagenicity test was determined by counting the cells harvested at the subcultivation during the expression period.

Result : No appreciable toxicity was observed under any treatment conditions, as determined from the number of cells harvested at the subcultivation during the expression period (>80% of control value).

In the presence of S9 mix, there was a clear mutagenic response.

At the highest concentration, 100 ug/ml, precipitation was observed.

Attachment :

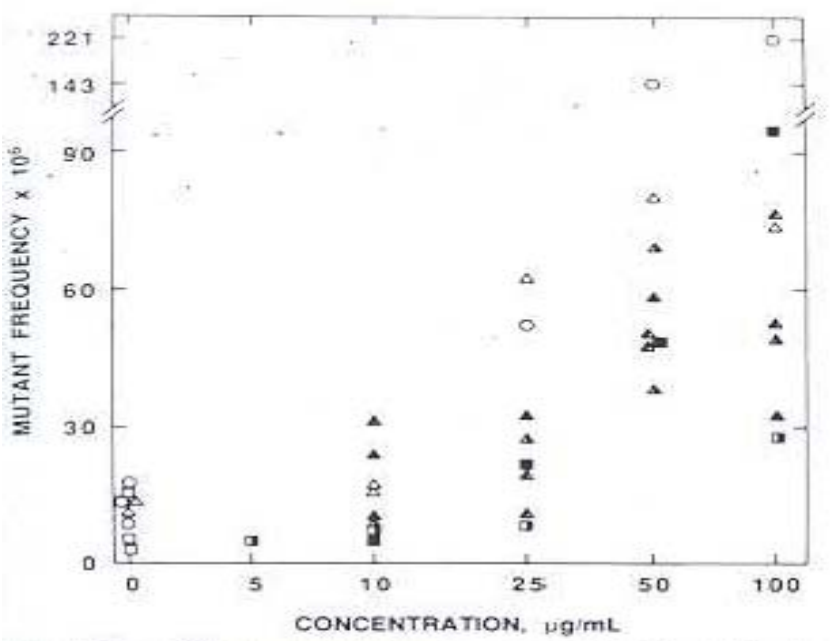


Fig. 1. Liver S9 mix-mediated mutagenicity of biphenyl (open symbols; also used for negative controls), 2-fluorobiphenyl (left half-solid symbols), 3-fluorobiphenyl (right half-solid symbols) and 4-fluorobiphenyl (solid symbols) in V79 cells. Each point represents a separate culture and is based on 6×10^6 cells subjected to selection. Different symbol shapes (circles, triangles, squares) indicate that the experiments were carried out on separate occasions. Biphenyl and 3-fluorobiphenyl precipitated at the highest concentration used (100 µg/ml), 2- and 4-fluorobiphenyl at the 2 highest concentrations (50 and 100 µg/ml). No appreciable toxicity was observed under any treatment conditions, as determined from the number of cells harvested at the subcultivation during the expression period (> 80% of control value).

Remark : The authors reported precipitate was observed at the highest concentration, 100 ug/ml. Precipitation, that was not visible, may have occurred at lower concentrations.

Centrifugation of the culture would not remove suspended particles from the cells. Therefore the dosing period was much longer than the 2 hour time period the authors suggest.

The cloning efficiency was not reported. If the cloning efficiency was much lower than the controls, significantly higher mutant frequencies could be observed. The available data is insufficient to adequately determine whether the cloning efficiency was affected.

Reliability : (4) not assignable
4e: Documentation insufficient for assessment

14.11.2005 (36)

Type : Mammalian cell gene mutation assay
System of testing : Mouse lymphoma L5178Y thymidine kinase locus assay
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result :
Method : other: essentially followed OECD 476 in vitro mammalian gene mutation assay
Year :
GLP : no data
Test substance :

5. Toxicity

Id 92-52-4
Date 02.05.2005

Method : Heterozygous L5178 TK+/- cells were grown in Fischer's medium containing 10% horse serum with additions. The pH of the culture medium was adjusted to 7.2 to improve the growth rate.

Liver homogenates (S9) were prepared from Aroclor 1254 pretreated male Sprague-Dawley rats.

Following the 4 hour treatment with the test material, the cells were resuspended in Fischer's medium containing 10% horse serum. The cells were allowed a 48-hour expression period.

The spontaneous mutation frequency was 76 +/- 25 without and 86 +/-33 x 10(6) cells with metabolic activation (n = 35 and 20), respectively.

The number of colonies formed on the six replicate control agar plates and the three replicate plates from each treated culture was tested for normal distribution according to Shapiro and Wilk (1965) and found to be normally distributed in 92% of the cases (n = 383). Further, the replicates were subjected to analysis of variance which showed that the variance of the control and treated replicates was equal in 95% of the comparisons (n = 317). Therefore, a pairwise two-tailed Student's t-test was performed on each set of treated replicates versus the corresponding solvent control replicates.

The authors also used the criteria requiring a 2-fold or greater increase in mutation frequency at 10% or higher total growth for a positive result (Clive et al., 1979).

References:
Clive, D., Johnson, K.O., Spector, J.F.S., Batson, A.G. and Brown, M.M.M. (1979). Validation and characterisation of the L5178Y TH+/- Mouse Lymphoma mutagen assay system. Mutation Research 59:61-108.

Shapiro, S.S. and Wilk, B.M. (1965). An analysis of variance test for normality (complete samples). Biometrika 52:591-611.

Result : Although positive results were observed with and without metabolic activation which were statistically significant, when one applies the criteria of Clive et al., 1979, biphenyl was negative in the assay without metabolic activation and was positive only at the two highest concentrations, 4 x 10(-5) and 6 x 10(-5).

Attachment :

J.Wangenheim and G.Bolesfoldi

Table I. Continued

Test compound	S9[a]	Concentration (mol/l)	Total growth[b]	Mutation frequency[c]	Mutation index[d]
Biphenyl[g]	–	0		66	
		0		57	
		0.987×10^{-4}	77	73	1.2
		1.970×10^{-4}	49	60	1.0
		2.960×10^{-4}	21	79*	1.3
		3.450×10^{-4}	14	105***	1.7
		3.950×10^{-4}	6	123***	2.0
	+	0		97	
		0		81	
		0.501×10^{-5}	102	93	1.0
		1.000×10^{-5}	75	98	1.1
		2.000×10^{-5}	35	123*	1.4
		4.000×10^{-5}	15	185***	2.1
		6.000×10^{-5}	12	319***	3.6

Remark : At the concentrations resulting in a mutation index greater than 2.0, the total growth was between 12 and 15%, indicative of severe toxicity to the surviving cells.

4 x 10(-5)M is equivalent to 6 ug/ml. Although this concentration is below that reported by Glatt to cause precipitation, 100 ug/ml, it's possible that biphenyl precipitate was present. If present, biphenyl may not have been removed during centrifugation and thus exposure may have been much longer than reported.

Reliability : (2) valid with restrictions

2e: Meets generally accepted scientific standards, well-documented and acceptable for assessment

15.11.2005 (37)

Type	:	other: DNA strand breaks
System of testing	:	Mouse lymphoma L5178Y TK+/- cells
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method :

Exponentially growing cells were labelled overnight with 0.2-0.3 uCi (methyl-3H)thymidine per ml culture. After 16-18 hours the culture was centrifuged, the radioactive medium was discarded and the cells washed with Hanks' balanced salt solution (HBSS), resuspended and incubated at 37C for 1-2 hours in medium without radioactive isotope. S9 mix or Fischer's medium without serum (Fop) without S9 mix was then added, after which exposure was initiated by adding biphenyl. The final cell concentration was approximately 2 x 10(5) cells/ml and the serum concentration 3% during exposure. Chemicals were dissolved in Fop, water, ethanol or dimethylsulphoxide at 100 times the greatest concentration to be tested, and appropriate dilutions made. If the solubility was too low to make a 100 times concentrated solution, a concentration 5 times that to be tested was made in Fop, with appropriate modifications of the test procedure to obtain the same cell density during the incubation. After 3 h exposure, 0.5 ml samples were set aside for viability estimation and the remaining 2.0 ml of cells were centrifuged and washed with HBSS to terminate the exposure.

All compounds with a few exceptions, were tested to concentrations that were clearly toxic to the cells. The exceptions were chemicals of low solubility or low toxicity.

Strand breaks were detected by alkaline unwinding and hydroxyapatite elution, according to Ahnstrom and Erixon (1981). Briefly, cells were lysed in an alkali solution. Samples were injected on columns of hydroxyapatite to separate ssDNA and dsDNA. The relative DNA content from the ssDNA and the dsDNA were measured based on the level of radioactivity present in each fraction.

Viability of the cell samples was determined by measuring the percentage of cells that did not uptake trypan blue. To minimize the underestimation of viability, due to prolonged exposure to the test substance during counting, cells were counted in the order of decreasing test compound concentration. Control cells were counted last.

Criteria used for classification:
1. An increase in the relative fraction of ssDNA of 6.5% at a relative toxicity of less than 5% is considered positive.
2. An increase in the relative fraction of ssDNA that is greater than the increase in the relative toxicity at the corresponding concentrations of the test compound at relative toxicities of 5% to 50% is considered positive if this occurs at 2 or more concentrations. If such an increase is seen at one concentration the result is classified as equivocal.
3. The classifications under 1 and 2 are true if the increase in relative fraction of ssDNA is dose-related.
4. A result is considered negative if a toxic response is obtained and no increase in the fraction of ssDNA is seen or if the increase is smaller than

the corresponding increase in relative toxicity. If toxicity is not evident the result cannot be adequately evaluated.

Result : Biphenyl was negative without S9 at concentrations as high as 5.0 x 10(-4)M. With S9, biphenyl was positive at concentrations of 0.5, 1.5 and 5.0 x 10(-4)M but was negative at 15.0 x 10(-4).

Attachment :

TABLE 1 (continued)

Test compound	S9 [1]	Concentration (mole/l)	Relative toxicity [2] (%)	Relative fraction ssDNA [3] (%)	Rel. frac. ssDNA [4] / Rel. tox.
Biphenyl [6]	–	0	0 (97)	0 (10.7)	
		0.500×10^{-4}	2	−1.3	
		1.50×10^{-4}	22	7.0	–
		5.00×10^{-4}	96	60.2	
	+	0	0 (96)	0 (14.7)	
		0.500×10^{-4}	1	23.8	+
		1.50×10^{-4}	7	35.0	+
		5.00×10^{-4}	23	43.0	+
		15.0×10^{-4}	93	57.0	

Remark : Based on the report by Glatt et al., 1992, a concentration of 100 ug/ml caused precipitate to form. The highest concentration in this study was 15.0 x 10(-4) which is equivalent to 210 ug/ml. This should have caused precipitate to form. The next lower concentration examined, 5.0 x 10(-4) which is equivalent to 77 ug/ml, may also have resulted in precipitate to form.

The authors indicated that they dissolved the test material at concentrations at least 5x higher than the highest concentration examined. However, based on Glatt's paper, the highest concentration is above the solubility limit for biphenyl. Since no analytical measurement was made of the dosing solution, it is unclear what concentration the animals were dosed with. The results of this study therefore have to be suspect.

The concentrations used in this study were at levels known to produce toxicity as reported by Wangenheim and Bolcsfoldi, 1988, when cells were allowed a 48 hour expression period. Since this study did not allow an expression period, cell death may not have been manifested, however severe toxicity was induced as exhibited in the thymidine kinase assay and thus the results of this single strand assay are questionable.

This is not a guideline study.

Reliability : (3) invalid
3a: Documentation insufficient for assessment

15.11.2005 (38)

Type : Gene mutation in Saccharomyces cerevisiae
System of testing :
Test concentration : 10(-5) and 10(-3) Molar
Cycotoxic concentr. : 10(-5) Molar
Metabolic activation : with and without
Result :
Method : other: Conducted prior to adoption of OECD 480
Year : 1986
GLP : no data
Test substance :

Method : Yeast cells from log-phase cultures were exposed to test agents (diphenyl and DMSO plus additional chemicals) with S9 and activation cofactors (NADP 1 mM, G-6-P 6 mM, MgCl2 4 mM, G-6-PDH 0.7 U/ml). In the experiments without metabolic activation, phosphate buffer, pH 7.4, was substituted for liver homogenate and cofactors. Every experiment was performed in triplicate. Cells were incubated at 37C for 4 hours in a roller drum. The cultures were plated on selective media for enumeration of ilv1+ revertants and trp+ convertants, and on a complete medium for survivor counts.

Based on the information provided, only two concentrations were tested, 10(-5) and 10(-3) Molar.

The S-9 preparation was obtained from male mice (Swiss albino). They were treated ip with sodium phenobarbital (100 mg/kg, half dose at the second day) for three days prior to being killed (24 hours after final injection) and with 80 mg/kg of B-naphtoflavone at the second day (48 hours before the sacrifice). Mouse livers were removed, homogenized in 4 volumes of 0.01M Na+/K+ phosphate buffer, pH 7.4 with 1.15% (w/v) KCl at 0 - 4C. The homogenate was centrifuged at 90000 ppm for 20 minutes at 0 to 4C. The supernatant was pipetted off and used immediately.

Test Substance : Biphenyl was purchased from Merck, West Germany. No purity information was provided.

Result : When diphenyl was directly suspended in medium (after shaking in water) even a nominal concentrations as high as 10(-3)M was ineffective in inducing any significant effects apart from a moderate toxicity. After dissolving DP in DMSO, diphenyl displayed evidence of toxicity. The genetic action of diphenyl was expressed on mitotic recombination, as well as on trp+ gene conversion and ilv+ reversion. The activity of diphenyl was evident also without metabolic activation, though the addition of the S-9 fraction enhanced the effects, especially trp+ gene conversion.

Attachment :

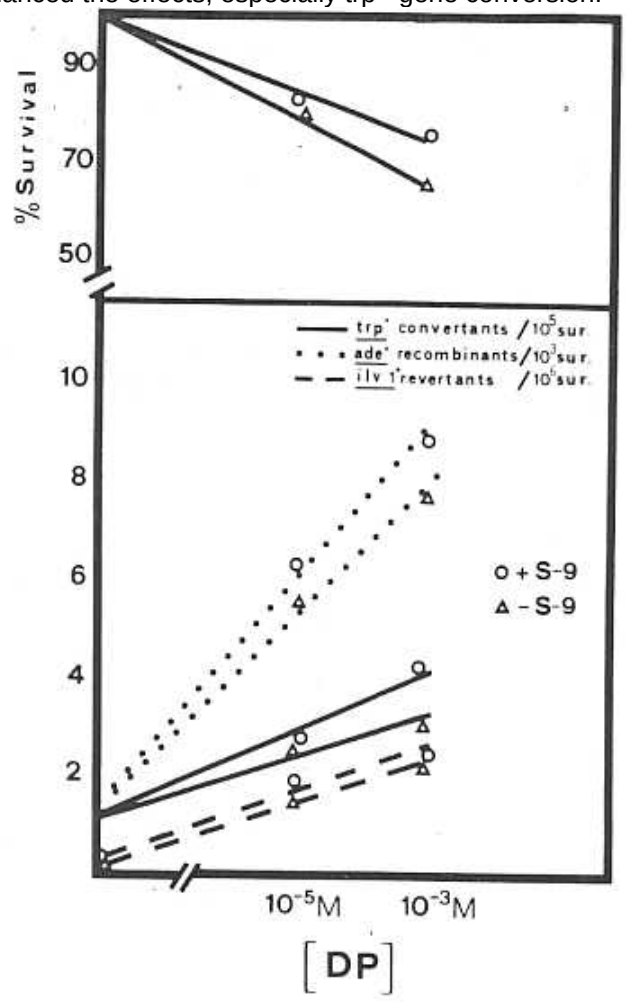


Fig. 12. Yeast. Induction of mitotic recombination, *trp*$^+$ gene conversion and *ilv 1*$^+$ reversion by DP.

Remark : For the two concentrations examined a decrease in the survival greater than the 5-10% currently recommended in the guidelines was observed. Therefore this study is considered to be unacceptable.

Reliability : 3
3b: Significant methodological deficiencies

15.11.2005 (39)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	
Strain	:	
Route of admin.	:	
Exposure period	:	
Doses	:	
Result	:	negative
Method	:	
Year	:	
GLP	:	
Test substance	:	

Method	:	No methods provided.
Remark	:	We have been unable to obtain any additional details on the method used or the results.
Result	:	Negative

02.05.2005 (40)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	gavage
Exposure period	:	administered on two consecutive days
Doses	:	200, 400 and 800 mg/kg/day
Result	:	negative
Method	:	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year	:	1997
GLP	:	yes
Test substance	:	other TS

Method : RANGE-FINDING Study: In phase I of the range-finding test, groups of mice (four/sex/dose) were treated with 1000 and 2000 mg/kg bw/day. Corn oil was used as the vehicle to administer the test material. Due to the thick suspension formed at concentrations necessary to achieve 2000 mg/kg/day and it's complete solubility at half that dose, a split dosing regimen (two administrations within a 2 hour period) was used to achieve the targeted doses (both 1000 and 2000 mg/kg/day) in phase 1. The treated animals were observed for up to 72 hours for clinical signs of toxicity, body temperature and/or death. At the 2000 mg/kg bw/day dose, all four female mice were declared moribund and two of the male mice were either declared moribund or spontaneously died prior to the second day of dosing. In both males and females at this dose level, there were significant decreases in body temperature (approximately 6.8ºC in males and 12.8ºC females) for 5 or more hours following the initial day of dosing. At the 1000 mg/kg bw/day dose level, all animals of both sexes survived to the end of the 72-hour observation period, except one male, which died spontaneously prior to the second day of dosing. No significant changes in body weights were observed in male or female mice at this dose level. In both males and females, there were significant decreases in the body temperature (approximately 4ºC) following the first treatment with the test material at the 1000 mg/kg bw/day dose level. These changes persisted for extended periods (= 5 hours) of time. Based upon these results, it was

concluded that the 2000 mg/kg bw/day exceeded the maximum tolerated dose (MTD).

In phase II, dose levels of 500, 750, and 1000 mg/kg bw/day were administered to male and female mice. In this phase, the test material was administered as a single bolus dose by oral gavage at a dosing volume of 10 ml/kg bw. The treated animals were observed for up to 72 hours for clinical signs of toxicity, body temperature and/or death. In the 1000 mg/kg bw/day group, two out of the four male mice spontaneously died after the first day of dosing. Based upon this, it was concluded that this dose level exceeded the MTD for males and further dosing of the surviving males was discontinued. All female mice in the 1000 mg/kg bw/day survived to the end of the observation period; however, decreased activity was observed among these animals. No significant changes in body weights were observed in the female mice at this dose level. In both sexes at this dose level, there were significant decreases in the body temperature (6-8oC) that persisted for extended periods of time (= 5 hours) after the first day of dosing. At the 750 mg/kg bw/day dose level, all animals survived to the end of the 72-hour observation period with no significant changes in body weight. Again, there were significant decreases in body temperature (approximately 3.5oC in males and 5.8oC in females) 5 hours after the initial dose. At the 500 mg/kg bw/day dose level, all animals survived and a decrease in body temperature of approximately 1oC was found in male and female mice.

DEFINITIVE Study: Based upon the results of the range-finding test, groups of male and female mice were administered 0, 200, 400, and 800 mg/kg bw/day of the test material on two consecutive days. Corn oil was used as the vehicle to administer the test material. Cyclophosphamide monohydrate (CP) was administered only once at a dose level of 120 mg/kg bw. There were six mice/sex/dose except in the 800 mg/kg bw/day group where an additional mouse was dosed as a possible replacement in the event of death occurring among the treated animals of this group. The mice were observed daily during the observation period for signs of toxicity, body temperature and/or death. Approximately 24 hours after the last dosing, bone marrow samples were collected from all animals.

The relative body temperatures of the treated animals were monitored during the range-finding test and the micronucleus study using programmable transponders (BioMedic Data Systems, Seaford, Delaware). At the end of the specified interval following treatment, the animals were euthanized and bone marrow samples were obtained from both femurs. Wedge smears were prepared on microscope slides using small portions of the cell suspension. The slides were allowed to air dry, fixed in cold methanol and stained with Wright-Giemsa.

All slides were coded and scored. Two thousand PCE were examined from each animal and the number of micronucleated polychromatic erythrocytes (MN-PCE) was recorded. The ratio of PCE to NCE in the bone marrow was determined in the micronucleus test by examining 200 erythrocytes. The ratio was expressed as PCE x 100/PCE+NCE.

Statistical Analysis: The raw data on the counts of MN-PCE for each animal was first transformed by adding one to each count and then taking the natural log of the adjusted number. The transformed MN-PCE data and the data on percent PCE were analyzed separately by a two-way analysis of variance (Winer, 1971). The sex by dose interaction in the two-way analysis was reviewed and if significant, a one-way analysis of variance was performed for each sex. Pairwise comparisons of treated vs. control groups was done, if the dose effect was significant, by Dunnett's t-test, one-sided (upper) for MN-PCE and two-sided for the percent PCE (Winer 1971). Linear dose-related trend tests were performed if any of the

pairwise comparisons yielded significant differences. The alpha level at which all tests were conducted was 0.05.

Evaluation Criteria: A test was considered valid if all of the following conditions were met:
The range of MN-PCE values in the negative controls were within reasonable limits of the recent (past five years) laboratory background range.
There was a significant increase in the incidence of MN-PCE in the positive control treatment as compared to the concurrent negative controls.
The mean for % PCE value in one or more of the test material treated groups was 20% of the control value indicating no undue effect on erythropoiesis (toxicity).

A test material was considered positive in this assay if the following criterion was met:
Statistically significant increase in MN-PCE frequency at one or more dose levels accompanied by a dose response.

A test material was considered negative in this assay if all of the following criteria were met:
No statistically significant dose-related increase in MN-PCE compared to the negative control.
A test result not meeting the criteria for either the positive or the negative response was considered to be equivocal.

Test substance : The purity of the test material was determined to be 99.6% ± 0.003% relative standard deviation corrected for water content (0.32% ± 8.3%) by gas chromatography and Karl Fisher coulometric titration with identification by gas chromatography mass spectroscopy and infrared spectroscopy.

Result : Based upon the decrease in body temperature observed at the 750 mg/kg bw/day dose level and the lethality at the 1000 mg/kg bw/day dose level, dose levels of 0, 200, 400, and 800 mg/kg bw/day biphenyl FP, were chosen for the main micronucleus study (MNT). Because of some indication of differences in toxicity between the sexes, both male and female mice were used in the MNT. The test material was administered as a single bolus dose on each day of dosing.

The treatments did not have a remarkable effect on the body weight of the animals. There were indications of toxicity upon daily observation during the in-life portion of the micronucleus test at the highest dose of 800 mg/kg bw/day in males and female mice. Decreased activity, crouched posture, and cold to touch were among the observations noted at this dose level. Accidental deaths caused by oral gavage errors occurred in a negative control male mouse and a male high dose mouse. At the highest dose of 800, mg/kg bw/day, a significant decrease in body temperature of approximately 6.4oC in males and 8.5oC in females was observed at 2 hours after the initial dose. Decreases in body temperature persisted for at least 5 hours; however, they recovered prior to the second day of dosing. The remarkable drops in temperature did not occur on the second day of dosing. At the 400 and 200 mg/kg bw/day dose levels, there were minimal to no decreases in body temperature.

There were no significant differences in MN-PCE frequencies between the groups treated with the test material and the negative controls in either male or female mice and all values were within laboratory historical control data (Tables 35 and 36). The adequacy of the experimental conditions for the detection of induced micronuclei was ascertained from the observation of a significant increase in the frequencies of micronucleated polychromatic erythrocytes in the positive control group.
The percent PCE values observed at the 800 mg/kg bw/day of biphenyl FP and the positive control dose were significantly lower than the negative control values.

Attached Document : EVALUATION OF BIPHENYL FP IN THE MOUSE BONE MARROW MICRONUCLEUS TEST

TABLE 35. Summary Micronucleated Polychromatic Erythrocytes (MN-PCE) Frequencies and % Polychromatic Erythrocytes (%PCE) – Males

TEST MATERIAL: Biphenyl

DOSE MG/KG		MN-PCE	PERCENT PCE
0[b]	MEAN	0.6	63.0
	S.D.	0.8	6.9
	N=[a]	5	5
200	MEAN	1.1	62.0
	S.D.	0.7	6.9
	N=	5	5
400	MEAN	1.4	60.0
	S.D.	0.9	2.3
	N=	5	5
800	MEAN	1.0	57.4[d]
	S.D.	1.0	2.4
	N=	5	5
CP 120[c]	MEAN	53.8[d]	42.4[d]
	S.D.	12.0	10.7
	N=	5	5

[a] N is the number of animals per dose group at the time of scheduled sacrifice. 2000 PCE were examined/animal for MN incidence, and expressed as MN/1000 PCE (‰MN-PCE).
[b] Mice were dosed with the vehicle (corn oil).
[c] CP = Cyclophosphamide monohydrate (positive control).
[d] The values are significantly different from the negative control (alpha=0.05).

EVALUATION OF BIPHENYL FP IN THE MOUSE BONE MARROW MICRONUCLEUS TEST

TABLE 36. Summary Micronucleated Polychromatic Erythrocytes (MN-PCE) Frequencies and % Polychromatic Erythrocytes (%PCE) – Females

TEST MATERIAL: Biphenyl

DOSE MG/KG		MN-PCE	PERCENT PCE
0[b]	MEAN	1.5	67.3
	S.D.	0.4	5.2
	N=[a]	5	5
200	MEAN	1.2	65.3
	S.D.	1.0	2.5
	N=	5	5
400	MEAN	1.1	63.5
	S.D.	0.7	6.1
	N=	5	5
800	MEAN	1.0	50.8[d]
	S.D.	0.8	3.8
	N=	5	5
CP 120[c]	MEAN	64.5[d]	50.3[d]
	S.D.	14.6	5.0
	N=	5	5

[a] N is the number of animals per dose group at the time of scheduled sacrifice. 2000 PCE were examined/animal for MN incidence, and expressed as MN/1000 PCE (‰MN-PCE).
[b] Mice were dosed with the vehicle (corn oil).
[c] CP = Cyclophosphamide monohydrate (positive control).
[d] The values are significantly different from the negative control (alpha=0.05).

Conclusion	:	Treatment-related toxicity was observed in male and female mice administered two consecutive daily doses of biphenyl FP at a dose of 800 mg/kg/day as determined by a decrease in body temperature as well as a significant reduction in the percent PCE. These results suggest that the test material was systemically available following oral gavage. Furthermore, the bioavailability of orally administered biphenyl FP has been demonstrated in the rat by Meyer and Scheline (1976). Based upon the results of the study reported herein, it was concluded that the test material, biphenyl FP, did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when given as a single oral dose on two consecutive days to male and female CD-1 mice. Hence, biphenyl FP is considered negative in this test system under the experimental conditions used. Reference: Meyer, T. and R. Scheline (1976). The Metabolism of Biphenyl. II. Phenolix Metabolites in the Rat. Acta Pharmacol. Et Toxicol. 39, 419-432.
Reliability	:	(1) valid without restriction 1a: GLP guideline study

27.02.2007 (41)

5.7 CARCINOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344/DuCrj
Route of admin.	:	oral feed
Exposure period	:	105 weeks
Frequency of treatm.	:	daily
Post exposure period	:	
Doses	:	0, 500, 1500 and 4500 ppm
Result	:	
Control group	:	yes, concurrent no treatment
Method	:	OECD Guide-line 453 "Combined Chronic Toxicity/Carcinogenicity Studies"
Year	:	1981
GLP	:	yes
Test substance	:	

Method :

A chronic study using F344/DuCrj rats was performed according to OECD 453 guideline. Groups of 50 male and 50 female rats were given the control diet or the biphenyl-containing diets throughout the 105-week period, starting at the age of 6 weeks. Dietary concentrations of biphenyl were 500, 1500 or 4500 ppm (0, 38, 113, or 338 mg/kg body weight per day).

Body weight and food consumption were measured once a week for the first 14 week of the 105-week study period and every 4 week thereafter. Urinary parameters of all surviving rats, including pH and occult blood, were examined with Urolabsix (Diagnostic Diutsior, Bayer, Elkhard, Germany) in the final week of the 105 week study period. All organs were examined macroscopically, selected organs, including gonads, weighed and the tissues for microscopic examination included the ones specified in the OECD test guidelines, and were fixed in 10% neutral buffered formalin, embedded in paraffin and 5um thick sections of all tissues and tumors were made and stained with hematoxylin and eosin.

All organs and tissues were preserved for microscopic examination. Based on the guideline (not defined in the publication) this included the following organs and tissues: brain* (medulla/pons, cerebellar cortex, cerebral cortex), pituitary, thyroid (including parathyroid), thymus, lungs (including

trachea), heart, salivary glands, liver*, spleen, kidneys*, adrenals*, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes, pancreas, gonads*, uterus, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord (cervical, thoracic, lumbar), sternum with bone marrow and femur (including joint) and eyes.

The incidence of neoplastic lesions was statistically analyzed by Fisher's exact test.

* Organs from 10 animals/sex/dose level for rodents were weighed.

Test substance : Biphenyl, CASNO 92-52-4, with a purity of >98% was obtained from Wako Pure Chemical Industries, Tokyo, Japan.

Result : Clinical Observations:
Body weights of rats fed 4500 ppm biphenyl in the diet were significantly decreased compared to control values but there was no statistical difference in the body weight between the 500 or 1500 ppm rats of both sexes and the corresponding controls (Fig 1).

Survival rates of all biphenyl-exposed groups except the 4500 ppm males were not statistically different from those of corresponding controls (Fig 2). Nineteen 4500 ppm males died during the 105-wk period. Their deaths were attributed primarily to the bladder tumors and the hematuria.

Thirty two males with clinical hematuria were observed and of the 32 males, 14 had anemia-colored skin and/or eyes in the 4500 ppm group. The hematuria first appeared around the 40th week of biphenyl exposure and continued thereafter with intermittent recoveries. The biphenyl exposed females had no clinical signs relating to biphenyl exposure.

Urinalysis:
The urinary pH significantly increased in the 4500 ppm males (Table 1). The incidence of positive occult blood significantly increased in the 4500 ppm rats of both sexes, and this was consistent with the above mentioned incidence of hematuria in the 4500 ppm males, but the number of females with positive occult blood was smaller than that of the males.

Organ weight:
A statistically significant increase in relative kidney weight was evident in the 1500 and 4500 ppm rats of both sexes and absolute kidney weight significantly increased in the 4500 ppm males (data not shown in publication).

Gross findings:
Bladder calculus was formed predominantly in male rats (Table 2): Forty three 4500 ppm males had bladder calculi, whereas only eight 4500 ppm females had calculi. No bladder calculus was found in any rats of either sex exposed to 500 or 1500 ppm biphenyl. Necropsy of dead and moribund animals revealed bladder calculi first appeared around the 40th week of the exposure together with the occurrence of hematuria.

Histopathologic findings:
Non-neoplastic lesions were limited to the urinary tract (Tables 2 and 3). Transitional cell hyperplasia, squamous cell hyperplasia and squamous cell metaplasia were observed in the urinary bladder of the 4500 ppm group. Hyperplasias were not diffusely distributed over the entire area of the bladder epithelium but developed in the focal area. The transitional cell hyperplasia was further classified into simple, nodular and papillary hyperplasia according to the histologic proliferation patterns by IARC and the Standardized System of Nomenclature and Diagnostics Criteria. The incidences of simple, nodular and papillary hyperplasia were 24%, 80% and 24% in the 4500 ppm males and 2%, 10% and 8% in the 4500 ppm

females, respectively, indicating that the simple hyperplasia occurred less frequently than the nodular and papillary hyperplasias. The simple hyperplasia was almost always accompanied by either nodular or papillary hyperplasia in the males. Ten 4500 ppm males had polyps in the bladder epithelium. The polyps observed in the present study were composed of abundant spindle cells that were proliferated around the transitional epithelial cells accompanied by inflammatory infiltration of the submucosal bladder epithelium, and were classified as the inflammatory type according to the Pathology of the Fischer rat. The polyps were accompanied by squamous metaplasia on their surface, and found at different loci from the bladder tumors.

In the ureters, the incidences of simple transitional cell hyperplasia and dilatation lumen were greater in the 4500 ppm males than in the corresponding females. In the renal pelvis, simple and nodular hyperplasia occurred frequently not only in the 4500 ppm males but also in the females exposed to 1500 and 4500 ppm. In the kidneys, statistically increased incidences of mineralization of cortico-medullary junction in the 4500 ppm males and mineralization of papilla in the 4500 ppm males and females were noted, whereas papillary necrosis, infarct and hemosiderin deposition occurred predominantly in the females.

A chronic study using F344/DuCrj rats, performed according to standard protocols, showed a significant increase in neoplastic and non-neoplastic lesions of the urinary bladder and, in high-dose males, a significant increase in calculi within the urinary bladder. In this 104 week study, dietary concentrations of Biphenyl were 0, 500, 1500, or 4500 ppm

A dose-dependent increase in hyperplasia of the renal pelvis epithelium was reported. Histopathological findings for the kidneys and urinary bladder are summarized in the following table. Other findings included increased serum levels of alkaline phosphatase, aspartate transaminase, and alanine transaminase activity and an increased urea nitrogen level in low-dose males and mid-dose females, which became more pronounced with increasing doses. Hematological effects were reported in mid- and high-dose females and in high-dose males. The No-Observed-Effect-Level (NOEL) for cancer in this study was 1500 ppm (113 mg/kg/day).

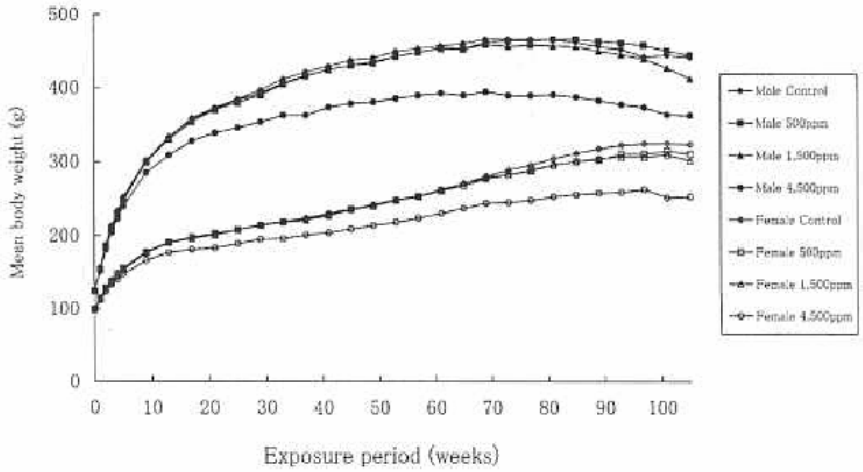
Summary of Effects:

High dose: Transitional cell carcinoma in males, bladder hyperplasia in males and females, kidney hyperplasia and mineralization in males and females, clinical chemical and hematological changes in males and females, increase in urea nitrogen in males and females.

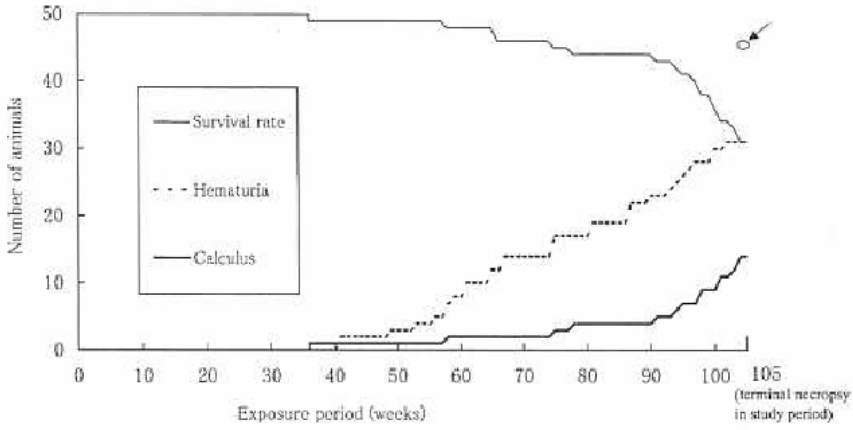
Mid dose: Kidney mineralization in males and females (minimal), increase in urea nitrogen (males and females), clinical chemical and hematological changes (males and females).

Low dose: Increase in urea nitrogen (males), clinical chemical and hematological changes (males).

Attached document : Figure 1 Time-course changes in mean body weight of male or female F344 rats exposed to 0, 500, 1500 or 4500 ppm biphenyl in the diet for 105 wk



Attached document : Figure 2 Time-course changes in the survival rate, the hematuria and the bladder calculi of the 4500 ppm males. The number of dead or moribund and necropsied animals with bladder calculi are indicated by a solid line and an open circle with arrow, respectively. The dotted line indicates cumulative number of animals showing hematuria



Attached document : Table 2 Incidences of urinary bladder lesions in male and female F-344 rats exposed to 0, 500, 1500 or 4500 ppm biphenyl in the diet for 105 wk

Sex of animal	Male				Female			
Group and the dose of chemicals administrated (per os)	Control	500 ppm	1,500 ppm	4,500 ppm	Control	500 ppm	1,500 ppm	4,500 ppm
Number of rats examined	50	50	50	50	50	50	50	50
transitional cell hyperplasia								
simple hyperplasia[a]	0	0	0	12[c]	0	0	1	1
nodular hyperplasia[a]	0	0	0	40[c]	1	0	0	5
papillary hyperplasia[a]	0	0	0	17[c]	0	0	0	4
total transitional cell hyperplasia	0	0	0	45	1	0	1	10
transitional cell papilloma	0	0	0	10[b,d]	0	0	0	0
transitional cell carcinoma	0	0	0	24[b,d]	0	0	0	0
total number of bladder tumors	0	0	0	31	0	0	0	0
squamous metaplasia[a]	0	0	0	19[c]	0	0	0	4
squamous cell hyperplasia[a]	0	0	0	13[c]	0	0	0	1
squamous cell papilloma and carcinoma	0	0	0	1	0	0	0	0
inflammatory polyp[a]	0	0	0	10[c]	0	0	0	0
calculus[e]	0	0	0	43	0	0	0	8

Conclusion : The present finding that thirty (94%) out of the thirty two 4500 ppm males with hematuria had calculi in the bladder or kidneys can be taken to indicate that there is a causal relationship between hematuria and the formation of bladder calculi. Therefore, it can be inferred that the hematuria in the 4500 ppm males is brought about by sustained mechanical damage to the bladder epithelium caused by the calculi. The occurrence of the hematuria observed after the 40th week of the exposure of males to 4500 ppm biphenyl may be causally related to the bladder calculus grown to a size large (>0.3 cm) enough to mechanically damage

the bladder epithelium.

Experimental evidence of a mechanism underlying male predominance in calculus formation in the bladder of biphenyl-exposed rats has been published (Ohnishi et al., 2000). They demonstrated that the male bladder calculi were composed of potassium 4-hydroxy-biphenyl-osulfate (4-HBPOSK), whereas the female calculi were made of 4-hydroxy-biphenyl (4-HBP) and KHSO4 to which 4-HBPOSK was further hydrolyzed only in female urine, and that a series of irreversible and stable metabolic pathways from biphenyl to 4-HBPOSK resulted in formation of calculus in the male rat, whereas calculus formation was prevented by reversible hydroxylation of 4-HBPOSK in female urine. The significantly increased urinary pH found in the 4500 ppm males may also facilitate the formation of bladder calculus, because coadministration of biphenyl and KHCO3 to male rats was reported to result in the formation of urine crystals of 4-HBPOSK through the increased urinary pH (Ohnishi et al., 2001). Therefore the present results showing that calculus formation as well as tumor induction occurred predominantly in the urinary bladder of the 4500 ppm males confirm the findings in the shorter term studies (Ohnishi et al., 2000; Ohnishi et al., 2001) and extends it to the experimental evidence that a close association between the bladder calculi and tumor induction exists in biphenyl-induced tumorigenesis.

Several reports have been published on a causal relationship between chemically induced bladder tumorigenesis and calculus formation. Fukushima et al., 1992, suggested that uracil induces bladder tumors through prolonged mechanical damage to bladder epithelium caused by large calculi. Melnick et al., 1984, reported that oral administration of 4500 ppm melamine in the diet to male rats significantly increased transitional cell carcinomas of the urinary bladder in significant association with bladder calculi. The present results show that biphenyl-induced bladder tumors occurred in close association with calculus formation and hematuria seems to be in favor of the mechanistic view that the formation of bladder calculi plays a critical role in bladder tumorigenesis (Fukushima et al., 1992). In addition, a very steep dose-response curve, that is, a high incidence of bladder tumors (60%) and hyperplasia (90%) in the 4500 ppm males, in sharp contrast with neither bladder tumors nor hyperplasia in the 1500 and 500 ppm-exposed males, may also be taken to indicate the existence of an exposure threshold above which the calculi are formed in the bladder, inducing bladder tumors through pre-neoplastic hyperplasia.

Reliability : (1) valid without restriction
1A GLP guideline study.

22.02.2005 (26)

Species	:	mouse
Sex	:	male/female
Strain	:	other: BDF1
Route of admin.	:	oral feed
Exposure period	:	104 weeks
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	667, 2000 or 6000 ppm
Result	:	
Control group	:	yes
Method	:	OECD Guide-line 453 "Combined Chronic Toxicity/Carcinogenicity Studies"
Year	:	1981
GLP	:	yes
Test substance	:	

Method : A chronic study using Crj:BDF1 mice of each sex was performed according to standard protocols. Groups of 50 mice of each sex were given diets containing 0, 667, 2000, or 6000 mg biphenyl/kg/day in the diet

(corresponding to 0, 97, 291 or 1050 mg/kg body weight/day in males and 0, 134, 414 or 1420 mg/kg body weight/day in females) for 104 weeks. At the end of the dosing period surviving mice were fasted overnight, blood samples collected for hematology and urinalysis determinations. The animals were sacrificed, examined for gross effects, selected organs were removed and weighed, tissues were removed, fixed, sectioned, stained with H&E and examined for microscopic changes.

All organs and tissues were preserved for microscopic examination. Based on the guideline (not defined in the publication) this included the following organs and tissues: brain* (medulla/pons, cerebellar cortex, cerebral cortex), pituitary, thyroid (including parathyroid), thymus, lungs (including trachea), heart, salivary glands, liver*, spleen, kidneys*, adrenals*, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, lymph nodes, pancreas, gonads*, uterus, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord (cervical, thoracic, lumbar), sternum with bone marrow and femur (including joint) and eyes.

Statistical analysis: Incidences of non-neoplastic lesions were analyzed by Fisher's exact test. Incidences of neoplastic lesions were statistically analyzed by Peto's trend test and Fisher's exact test. Body weight, food consumption, organ weight and hematological and blood biochemical parameters were analyzed by Dunnett's test.

* Organs from 10 animals/sex/dose level for rodents were weighed.

Test substance : Biphenyl, CAS# 92-52-4. Purity greater than 98% was obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan.

Result : Histopathological examination: Histopathological observations were confined primarily to the liver and kidney. Incidences of hepatocellular adenomas and combined incidences of hepatocellular adenomas and carcinomas were significantly increased in the females fed 2000 or 6000 ppm diets. The incidence of hepatocellular carcinomas was also significantly increased in the females fed 2000 ppm in the diet. Although 5 cases of hepatocellular carcinoma in females fed 667 or 6000 ppm in the diet were not statistically significant, the tumor incidence (10%) exceeded the historical control incidence (26 cases (2.5%) in 1048 female mice in 21 carcinogenicity studies, with the maximum incidence of 8%). Notably, incidences of hepatocellular adenomas and carcinomas were not increased in any of the males fed diets containing biphenyl.

Attached document :

Table 3. Incidences of gross and histopathological findings in the male and female mice fed diet containing biphenyl for two years

Sex of animal	Male				Peto's	Female				Peto's
Group (ppm)	Control	667	2,000	6,000	test	Control	667	2,000	6,000	test
Number of mice examined	50	49	50	50		50	50	50	49	
Gross finding										
Liver										
Nodule	20	16	14	11		7 (5)[c]	13 (5)	24 (16)	26 (19)	
Histopathological findings										
Liver										
Hepatocellular adenoma	8	6	7	3		2	3	12*	10*	↑
Hepatocellular carcinoma	8	8	5	4		1	5	7*	5	
Hepatocellular adenoma + carcinoma[a]	16	12	9	7		3	8	16**	14*	↑↑
Basophilic cell foci[b]	0	6**	1	2		1	1	12**	6*	
Clear cell foci[b]	0	6**	2	0		2	1	3	2	
Eosinophilic cell foci[b]	0	0	0	0		0	1	0	0	
Kidney										
Desquamation: pelvis[b]	0	0	0	10**		4	0	0	15**	
Mineralization in the inner stripe-outer medulla[b]	9	8	14	14		3	5	12*	26**	

* and **: Significantly different at $p<0.05$ and $p<0.01$, respectively, by Fisher's test.
↑ and ↑↑: Significantly different at $p<0.05$ and $p<0.01$, respectively, by Peto's test.
a) Combined incidence of hepatocellular adenoma and carcinoma.
b) Number of the histopathological finding with a different grade (slight, moderate, marked or severe) was summed.
c) The parenthesized value indicates the number of the animals bearing the liver nodule in which the proliferative lesion was histopathologically observed.

Remark : Body weights of male and female mice fed 6000 ppm in the diet were decreased 30 and 25%, respectively. The degree of body weight effects observed clearly exceed the maximum tolerated dose.

The Japan Bioassay Research Center (JBRC) has conducted 32 studies using the BDF1 strain of mice and included 1596 male and 1596 female controls (personal communication Y Umeda). The average incidence of spontaneous hepatocellular carcinomas in control male and female BDF1 mice was 19.9% and 2.4% in 32 carcinogenicity studies, respectively. The average incidence of hepatocellular carcinomas and adenomas combined was 34.9 and 7.9% in these same studies, respectively.

As discussed by Umeda et al., 2004, peroxisome proliferation was observed in the liver of female mice fed 6000 ppm biphenyl for 13 weeks. This was not observed in male mice in the same study.

At the end of the two year study, the relative liver weight for the high dose males was increased 12% from control values (see Table in Repeated Dose Toxicity). The absolute and relative liver weights of the low and middle dose males were comparable to control values. In females, the absolute liver weights were increased 18-25% in all treated groups while the relative liver weights were increased 29-61%.

In a subacute study, Sunouchi et al., 1999, reported slight increases in KCN-insensitive palmitoyl CoA oxidation in liver homogenates and lauric acid 12-hydroxylation in liver microsomes in animals receiving 5.2 mmol/kg biphenyl (~800 mg/kg). The response observed was much lower than for the positive control, clofibrate, which is not generally considered a strong peroxisome proliferator. The dose levels used by Sunouchi were much lower than those used in the chronic study, it is unclear the extent of peroxisome proliferation that occurred at the higher doses.

The authors suggest peroxisome proliferation is induced by the formation of 2,5-dihydroxybiphenyl which is structurally similar to CI-924 a peroxisome proliferator. The presumption by the authors is that the first metabolite formed by the degradation of biphenyl is 2-hydroxybiphenyl. However, as reviewed by Bomhard et al., (2002), in chronic studies of 2-hydroxybiphenyl (also known as orthophenylphenol) peroxisome proliferation was not reported in male or female mice. Thus it is unclear whether the parent or a metabolite induces peroxisome proliferation.

Reference
Bomhard, E.M., Brendler-Schwaab, S.Y., Freyberger, A., Herbold, B.A., Leser, K.H. and Richter, M. (2002). Critical Reviews in Toxicology 32:551-626.

Reliability : (1) valid without restriction
Modern guideline study under GLP's with sufficient documentation

10.06.2005 (8)

5.8.1 TOXICITY TO FERTILITY

Type : other: Three generation study
Species : rat
Sex :
Strain : Long-Evans
Route of admin. : oral feed
Exposure period : lifetime
Frequency of treatm. : Cont
Premating exposure period
Male :
Female :
Duration of test :

No. of generation studies	:	3
Doses	:	100, 1000 or 10000 ppm
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 1000 ppm
NOAEL F1 offspring	:	= 1000 ppm
NOAEL F2 offspring	:	= 1000 ppm
Result	:	Not Specific Reproductive Toxin
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method :

In this multigeneration test, weanling Long Evans rats of each sex were raised on a basal control diet until approximately four months of age at which time they were then divided into groups of three males and nine females each and fed the following diets:

Group 1: Control basal diet
Group 2: Basal diet containing 0.01% Biphenyl (100 ppm)
Group 3: Basal diet containing 0.1% Biphenyl (1000 ppm)
Group 4: Basal diet containing 01.0% Biphenyl (10,000 ppm)

For breeding, three females and-one male-were placed together in wire bottom cage. They were housed in air-conditioned animal quarters maintained at 73-77F and 45-50% relative humidity with diets and water available ad lib. Breeding females not observed to be pregnant after four weeks were placed with another male of the same group. If no pregnancy resulted after a total of nine weeks, the female was recorded as sterile. Females observed to be pregnant were placed in individual cages with nesting material. Litter size was recorded at birth.

At two days of age, the young were weighed and reduced to seven per litter. Pups were weaned at three weeks of age and weighed weekly from the third through the sixth week of life.

Young (Generation 2) from the first generation rats were continued after weaning on-the same diets that their parents had received. At ten weeks of age, nine females and three males of the second generation were mated. In turn, their offspring (Generation 3) were treated as above and in-turn they were mated to produce the fourth generation. Fourth generation rats were sacrificed at three weeks of age and twelve animals from each diet group autopsied for gross pathology.

No additional information provided.

Remark : Although feed consumption data and breeding rat weight data are not available, the hypothesis that the high-dose effects are related to reduced food consumption due to palatability is supported by the 1960 Ambrose feeding study where diets containing 5000 and 10000 ppm Biphenyl were shown to result in reduced food consumptions and reduced body weight gain.

Result : Rats that were maintained on diets containing 100 or 1000 ppm Biphenyl had a reproduction record entirely consistent with the control rats in respect to fertility, lactation, size of litter, growth and mortality of the pups. Reproductive performance through three generations of exposure showed no cumulative effect of treatment and all rats of the forth generation were unremarkable at sacrifice and necropsy.

Data for dams is as follows:

Days

DIET	Gen	Dams Bred	Litters Cast	Mating to littering
Control	1	9	8	24
	2	9	8	28
	3	9	8	26
100 ppm	1	9	8	32
	2	9	9	31
	3	9	9	31
1000 ppm	1	9	9	29
	2	9	9	28
	3	9	9	27
10000 ppm	1	9	6	33
	2	9	7	33
	3	9	8	31

Data for pups:

DIET	Gen	# Pups/ litter at birth	Mean weight pups (g) 2d	3w	4w	5w	6w	Mean Litter Size 3w	6w
Control	2	8.4	8.8	48	74	90	104	6.1	4.7
	3	7.3	7.7	45	70	95	122	6.6	5.0
	4	10.2	8.0	50				7.0	
100 ppm	2	8.6	7.0	50	59	88	110	6.4	5.6
	3	9.3	8.6	45	66	97	125	6.2	6.2
	4	11.3	8.1	44				7.0	
1000 ppm	2	7.0	8.3	47		81	87	5.7	5.4
	3	8.4	7.7	44	64	95	123	6.4	5.7
	4	8.3	8.6	46				5.6	
10000 ppm	2	5.7	8.6	35			64	5.0	4.2
	3	5.4	7.1	36	49	69	91	4.7	4.4
	4	7.4	7.0	32				6.5	

Administration of the 10,000 ppm Biphenyl diet proved to have adverse effects on reproductive parameters. Fertility of the females was decreased from an average of 8.3 litters from 9 females for the controls to 7.0 litters from the 10,000 ppm group. The mean litter size was significantly (statistically) smaller with an average of 8.6 pups/litter for controls and 6.2 pups/litter in the 10,000 ppm group. Body weights of pups fed diets of 10,000 ppm Biphenyl were statistically lower than control rats at both three and six weeks of age. All rats appeared normal at necropsy and there was no evidence of cumulative toxicity over the three generations studied.

It was suggested that the adverse effects on fertility may have been caused by unpalatability of the diet resulting in lower food consumption rather than by any effect of the test substance on physiological function.

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance : Biphenyl, CAS # 92-52-4.
Conclusion : Marginally reduced fertility occurred at feeding levels that were toxic to the young adult animals as manifest by reduction in weight gains. Feed levels that were not associated with parental toxicity did not have any effect on

reproductive parameters over four generations of exposure. Biphenyl is not a specific reproductive toxin to the rat

Reliability : (2) valid with restrictions
Although this study lacks some details and it was conducted by a scientifically defensible method and is considered to have good reliability. Another strength of the study is that there was a clear maternally and paternally toxic dose tested that produced only small effects on reproductive parameters

Flag : Critical study for SIDS endpoint

08.02.2005 (42)

Type	:	Fertility
Species	:	rat
Sex	:	male/female
Strain	:	
Route of admin.	:	oral feed
Exposure period	:	11 or 60 days before mating through weaning
Frequency of treatm.	:	Cont
Premating exposure period		
Male	:	11 or 60 days
Female	:	11 or 60 days
Duration of test	:	
No. of generation studies	:	1
Doses	:	1,000 or 5,000 ppm
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 1000 ppm
NOAEL F1 offspring	:	= 5000 ppm
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method : Groups of 15 weanling rats of each sex were placed on diets containing seven levels of biphenyl for a period of 750 days. For the two highest dose levels, pair-fed controls were also included to discriminate between compound-related effects and decreased feed consumption. In the main study, animals were housed 5 to a cage and had free access to food and water at all times. During the period of growth, rats were weighed and food consumption was determined weekly. Following the period of active growth, the rats were weighed at 50-day intervals for the duration of the study. Animals were examined at the time of weighing for gross evidence of tumors. At sacrifice, animals were necropsied, weights of liver, kidneys, heart, and testes were determined. Hematoxylin-eosin stained sections of heart, lung, liver, kidney, adrenal, spleen, pancreas, stomach, intestine, bladder, thyroid, brain, pituitary, and gonads were prepared and bone marrow smears of representative animals were prepared.

Dosed feed levels for the study were 0, 10, 50, 100, 500, 1,000, 5,000 or 10,000 ppm (0.001 to 1%).

Studies on possible reproductive effects and survival of young were also conducted as follows. Ten weanling female and five male rats were placed on control diet for 60 days, and subsequently mated, one male to two females. An identical experiment included Biphenyl at a dietary level of 0.1%. Nine female and 3 male rats were fed a dietary level of 0.5% Biphenyl in a subsequent study. All rats continued exposure until the pups of all litters were weaned.

In a second series of reproductive experiments, 90-day old rats were exposed for 11 days before mating and continuously until weaning of pups. Using this dosing schedule, 8 female and 4 male rats were placed on the

control diet, 8 females and 4 males received 0.1%, and 9 females and 3 males received 0.5% dietary levels of Biphenyl.

No additional information provided.

Result : Two studies of potential reproductive effects and survival of young were conducted. In the first, male and female animals were treated for 60 days pre-mating with diets containing 0, 5,000, or 10,000 ppm Biphenyl. Dams continued exposure until weaning of pups. The group sizes are shown in the results table

STUDY 1: 60-Day Pre-mating Treatment.

Conc	Females Mated	Females delivering	Total pups	Range of litter size	pups/ litter
0	10	9	59	3-9	6.5
5000	10	10	67	2-10	6.7
10000	9	8	53	3-9	6.6

In the second study, 90-day old rats of each sex were exposed for 11 days before mating and continuously until weaning of pups. The group sizes are shown in the results table

STUDY 2: 11-Day Pre-mating Treatment.

Conc	Females Mated	Females delivering	Total pups	Range of litter size	pups/ litter
0	8	8	64	5-13	8.0
5000	8	6	63	3-10	10.5
10000	9	8	48	3-9	6.0

Statistical analysis of reproductive data was not presented. It was concluded that "Dietary levels of 1000 and 5000 ppm Biphenyl had no significant effect on reproduction.

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance : Biphenyl, CASNO 92-52-4
Conclusion : No effect on reproductive ability or pup survival was found
Reliability : (2) valid with restrictions
Study limited in scope, information about fertility and pup survival valuable but not definitive due to lack of modern end-point parameters

08.02.2005 (25)

Type : Fertility
Species : rat
Sex : male/female
Strain : Fischer 344/DuCrj
Route of admin. : oral feed
Exposure period : 2 Years
Frequency of treatm. : Continuous
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 500, 1500 and 4500 ppm (38, 113, and 338 mg/kg-day)
Control group : yes, concurrent vehicle

Method : Two-year carcinogenicity studies were conducted using rats and mice of

each sex by the Japan Bioassay Research Center. In these studies rats were fed biphenyl in the diet at levels such that the average dose over the two-year bioassay was 38, 113, or 338 mg/kg-day for rats of each sex. Mice, likewise received biphenyl containing feed for a period of two-years at feed concentrations such that the dose levels were 100, 300 or 900 mg/kg-day. The initial group size for this study was 50 animals per sex for each dose level. The survival rate was high with approximately 80 % of male mice, 60% of female mice, 75% of male rats and 80% of female rats surviving.

The dosage levels were selected based on a subchronic evaluation in rats and mice and were set to represent the maximum-tolerated dose (MTD) to provide a robust test for carcinogenic potential of biphenyl. Information concerning the long-term effects of biphenyl on a variety of other organ systems is also obtained from the two-year bioassays because animals receive a "complete" necropsy, and an extensive and generally standardized list of tissues are examined by gross and microscopic means. The report containing the organ list for microscopic examination was not available for review but is can safely be assumed that the reproductive organs were given a through examination. This is confirmed in the WHO IPCS CICAD document in which the results of the two-year bioassay are presented in considerable detail and it is noted specifically that: "Histopathological changes within the male and female reproductive systems were not observed in rats or mice administered biphenyl at 400-4500 mg/kg in the diet for 2 years". In a modern guideline carcinogenicity study such as was conducted on biphenyl, the following reproductive organs are routinely microscopically examined in at least high-dose and control animals.

- epididymides
- mammary gland
- ovaries
- pituitary gland
- preputial glands
- prostate
- seminal vesicle
- testes
- thyroid
- uteru

Test substance : Biphenyl, CASNO 92-52-4, with a purity of >98% was obtained from Wako Pure Chemical Industries, Tokyo, Japan.

Conclusion : Administration of dietary concentrations of biphenyl to F344/DuCrj rats of each sex sufficient to cause frank organ toxicity in the bladder, kidneys and other organ systems did not result in any observable adverse effect on reproductive organ

Reliability : (1) valid without restriction
Guideline Study.

01.03.2005 (43)

Type : other: Chronic
Species : mouse
Sex : male/female
Strain : other: Cjr:BDF1
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatm. : Continuous
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :

Doses	:	100, 300 or 900 mg/kg-day
Control group	:	
Method	:	Two-year carcinogenicity studies were conducted using rats and mice of each sex by the Japan Bioassay Research Center. In these studies rats were fed biphenyl in the diet at levels such that the average dose over the two-year bioassay was 38, 113, or 338 mg/kg-day for rats of each sex. Mice, likewise received biphenyl containing feed for a period of two-years at feed concentrations such that the dose levels were 100, 300 or 900 mg/kg-day. The initial group size for this study was 50 animals per sex for each dose level. The survival rate was high with approximately 80 % of male mice, 60% of female mice, 75% of male rats and 80% of female rats surviving. The dosage levels were selected based on a subchronic evaluation in rats and mice and were set to represent the maximum-tolerated dose (MTD) to provide a robust test for carcinogenic potential of biphenyl. Information concerning the long-term effects of biphenyl on a variety of other organ systems is also obtained from the two-year bioassays because animals receive a "complete" necropsy, and an extensive and generally standardized list of tissues are examined by gross and microscopic means. In the case of biphenyl report the report containing the organ list for microscopic examination was not available for review but is can safely be assumed that the reproductive organs were given a through examination. This is confirmed in the WHO IPCS CICAD document in which the results of the two-year bioassay are presented in considerable detail and it is noted specifically that: "Histopathological changes within the male and female reproductive systems were not observed in rats or mice administered biphenyl at 400-4500 mg/kg in the diet for 2 years". In a modern guideline carcinogenicity study such as was conducted on biphenyl, the following reproductive organs are routinely microscopically examined in at least high-dose and control animals. - epididymides - mammary gland - ovaries - pituitary gland - preputial glands - prostate - seminal vesicle - testes - thyroid - uteru
Test substance	:	Biphenyl CASNO 92-52-4, purity > 99.1
Conclusion	:	Administration of dietary concentrations of biphenyl to Cjr:BDF1 mice of each sex sufficient to cause a reduction in body weight gain did not result in any observable adverse effect on reproductive organs.
Reliability	:	(1) valid without restriction Guideline study.

01.03.2005 (44)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	day 6-15 of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	125, 250, 500, 1000 mg/kg bw
Control group	:	yes, concurrent vehicle

NOAEL maternal tox.	:	= 500 mg/kg bw
NOAEL teratogen.	:	= 500 mg/kg bw
Result	:	Not specific developmental toxin in this study
Method	:	other: essentially follows OECD 414 study design adopted several years after this study was conducted
Year	:	
GLP	:	no data
Test substance	:	

Method :

Female Wistar rats, 175-200 g body weight, were paired overnight with proven males. The morning a positive vaginal smear was observed was counted as Day 1 of gestation. Eighteen to 20 mated females were assigned to each dosage group and a control group was included.

Females were weighed on the 1st, 6th through 15th, and 22nd days of pregnancy. At sacrifice on day 22 of gestation, the carcass was before and after the uterine contents were removed, the number of corpora lutea were determined, and a necropsy performed. The fetuses were weighed and examined for viability and external malformations. Early resorption or implantation sites and fetuses dying at a late stage in their development were recorded as dead fetuses. Two-thirds of the live fetuses from each litter were studied for skeletal development following alizarin red staining). The remaining fetuses were fixed in Bouin's fluid, sectioned at 1-mm intervals with a razor blade, and examined for visceral anomalies.

Test material was administered on days 6 through 15 of gestation by gavage using corn oil as vehicle. Dose levels were selected on the basis of a preliminary experiment in which dosed of 2000 mg/kg resulted in the death of all dams 2-3 days after initiation of dosing. The dosing volume 10 ml/kg body weight and the doses employed were 0, 125, 250, 500 or 1000 mg/kg.

Statistical methods. In assessing effects of treatment on maternal body weight, mean and SE were calculated for each experimental group and t values were obtained for test group versus control group differences in means. The litter was treated as the basic observational unit for analysis of fetal parameters, and the proportion of a litter having a particular effect was calculated. The mean and its SE of the proportion in the different test groups, were derived. The t test was used for comparison of test and control values and differences were considered to be significant at $p < 0.05$

Result :

Maternal Effects: In the animals receiving the highest dose, 1000 mg/ kg, it was found that resorption occurred in one litter, five animals were found not to be pregnant (which may have been due to interference with implantation), and mortality occurred in an additional five females. Each death occurred during the dosing period and was preceded by a sharp reduction in body weight and diarrhea. The remaining doses of biphenyl, 125, 250, and 500 mg/kg, elicited no signs of toxicity. Maternal body weights were only presented graphically in the publication. Examination of the graph indicates reduction in body weight gain only at the 1000 mg/kg level.

Fetal and Related Effects: at the 1000 mg; kg dose Biphenyl was lethal for five dams; however, in those that survived, it did not affect the incidence of corpora lutea, live fetuses, or dead fetuses plus resorption sites, nor did it affect fetal weight. Although fetal weight was reduced, and the incidence of dead fetuses plus resorptions increased, these values were not significantly different from control animals. In the 1000 and 500 mg/kg groups, there was a slight increase in the number of fetuses with missing and unossified sternebrae or with delayed calvarial ossification but these increases were not statistically significant.

EFFECT	DOSE LEVEL 0	125	250	500	1000
Number of rats with live fetuses at term/number mated	6/18	20/20	18/19	18/20	9/20
Number of corpora lutea per pregnancy	12.6±0.4	12.9±0.4	13.7±0.5	13.3±0.4	12.5±0.7
Number of live fetuses per pregnancy	11.3±0.7	11.8±0.6	11.9±0.6	11.2±0.5	10.7±1.3
Dead and resorbed fetuses	4.8	3.3	6.1	7.8	13.7
Fetal weight (g mean ±SE)	5.1±0.1	5.3±0.1	5.2±0.1	5.2±0.1	4.5±0.3
Number of anomalous fetuses	17/176	22/236	22/213	35/199	25/107
Number of anomalous litters	8/16	11//20	13/18	15/18	3/9
ANOMALIES (#fetuses affected)					
Wavy ribs, uni- and bilater	3	7	9	8	5
Extra ribs, uni and bilater	9	12	9	15	6
13th rib, small sized	1	1	2	1	0
Sternebrae, missing or unos	4	3	4	16	17
Calvarium, delayed ossifica	0	2	0	0	8
Miscellaneous	1	1	1	0	0

Source : Toxicology and Regulatory Affairs Freeburg, IL
Test substance : Biphenyl, CAS # 92-52-4 Technical grade material of >99.9% purity was used.
Conclusion : The maternal and fetal NOEL is 500 mg/kg. In spite of severe maternal toxicity at 1000 mg/kg, there was only minor fetotoxicty produced at this level. The test material did not have specific developmental effects in this study
Reliability : (2) valid with restrictions
Published reports are assigned a reliability of 2. Despite differences from the current guideline and the lack of details that would be reported in a modern investigation, the study appears to have been well conducted and the data appear to be robust
Flag : Critical study for SIDS endpoint
09.02.2005 (45)

Species : mouse
Sex : female
Strain : other: CLFP (ICI Strain 2) outbred
Route of admin. : gavage
Exposure period : day 6-15 of gestation
Frequency of treatm. : daily
Duration of test :
Doses : 125, 250, 500 or 1000
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 500 mg/kg bw
NOAEL teratogen. : = 500 - mg/kg bw
Result : Not specific developmental toxin
Method : other: EPA Guideline 83-3, OECD 414 Draft
Year : 1984
GLP : yes
Test substance :

Method : Groups of 40 female SPF CLFP (ICI Strain 2) outbred mice (weight range 26 to 42.9 grams) that has been time-mated to males of the same strain were treated by gavage with Technical Biphenyl in corn oil dosed from day 6 to 15 of pregnancy. Dose levels, selected based on a preliminary study, were 0, 125, 250, 500 or 1000 mg/kg body weight. Animals were weighed on day 1, 3, 6, 8, 10, 14 and 17 of pregnancy. Food consumption was determined as a function of the weighing intervals. Animals were sacrificed on gd day 17.5 cervical dislocation, dissected and examined for congenital

abnormalities and macroscopic pathological changes in maternal organs, the ovaries and uteri were examined immediately to determine: number and distribution of live young, number and distribution of embryofoetal deaths, individual fetal weights, fetal abnormalities.

Live young were examined externally and weighed. Half the fetuses in each litter were preserved is Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique. The remainder were fixed in 74-OP industrial methylated Sprit for subsequent macroscopic examination, evisceration, clearing and alizarin staining for skeletal examination. All fetuses were sexed by gonadal inspection following preservation.

Statistical Analysis: Statistical analysis were routinely performed on litter data using a two-tailed test for significance at the 0.05 level. Non-parametric tests are primarily used due to non-normal distributions of most parameters. Mean values of litter size, post-implantation loss, litter weight, mean pup weight and the incidence of anomalous offspring were analyzed by the Jonckheere and Kruskal-Wallis tests. Fisher's exact test was employed where a high incidence (75%) of tied values occurred. Incidence values for maternal mortality and total resorption were also analyzed using the Chi-
Square test

Remark : Based on the other developmental toxicity study, technical grade material is expected to be >99.9% pure.

Result : Maternal Effects: There was a high incidence of non-pregnancy in all groups (the reason for the large group size) and it was not related to the test material. In the animals receiving the highest dose, 1000 mg/kg, it was found that total resorption occurred in seven litters. This resulted in an overall reduction in maternal weight gain but no reduction if only animals bearing live pups are considered. Food consumption was similar in all groups and controls. Maternal mortality was increased at the high-dose level and reported as 0, 0, 1, 2, and 8 in control to high dose, respectively. No clear cause of death was discovered at necropsy of the decedents. No clear treatment-related effects were seen at terminal sacrifice.

	GROUP (mg/kg)				
	0	125	250	500	1000
Mated	40	40	40	40	40
Sacrificed	0	0	1	0	4
Died	0	0	0	2	4
Tot mortality	0	0	1	2	8**
Non-Pregnant	17	16	19	18	15
Total resorption	1	0	3	4	7**
With live young	22	24	17	16	10

** < 0.01 Chi Square Test

Litter Effects: Total resorptions were significantly increased in the high-dose group and the incidence was 1, 0, 3, 4 and 7, control to high dose. Mean litter size was also reduced at the high dose but this was entirely due to the 7 dams resorbing the entire litter. Mean litter and fetal weights were similar in all groups. Sex ratio was not affected by treatment.

Malformations: The incidence of malformed fetuses was 3, 6, 8, 3 and 4 from control to high-dose. Neither the type nor distribution suggested an association with treatment. Specific malformations are listed below

Variations: There were slight intergroup differences in mean incidence of fetuses with extra ribs or variant sternebrae but these were not suggestive of a treatment-related effect.

	DOSE LEVEL				
	0	125	250	500	1000
EFFECT					
Live pups/dam	13.3	15.2	11.4	10.9	8.6
Live pups/dam with live pups	13.9	15.2	13.4	13.6	14.7
Implants/dam	15.1	16.3	13.7	15.3	13.7
Postimplantation loss (%)**	12.5	7.0	25.8	28.6	46.6*
Sex ratio (% males)	53.9	50.4	39.3*	57.9	51.3
Fetal weight	0.99	0.99	1.04	1.07	1.00
Number of malformations	3/305	6/365	8/227	3/217	4/147
Number of anomalous fetuses***					
Visceral anomalies	14/149	16/179	13/109	12/105	3/70
Skeletal anomalies	18/153	24/180	9/110	7/109	2/73

* $p < 0.05$

** Postimplantation loss is calculated as the mean of the percent losses per individual litter.
*** Anomalous is synonymous with variation.

Malformations consisted of the following findings:

1000 mg/kg (4 pups)
-Small with interventricular septal defect and right azygous vein
-Interventricular septal defect
-Incomplete inferior vena cava and extra digit
-Umbilical hernia and fused costal cartilage elements

500 mg/kg (3 pups)
-Interventricular septal defect
-Small with thoracogastroschisis and retarded ossification
-Small with interventricular septal defect and hydrocephaly, etc.

250 mg/kg (8 pups)
-Double outlet rt. ventricle and interventricular septal defect
-Interventricular septal defect
-Small with craniofacial irreg and retarded ossification
-Umbilical hernia and partially fused costal cartilage elements
-Small with interventricular septal defect
-Incomplete inferior vena cava and displacement of adrenal
-Small with domed cranium and misshapen vertebrae
-Rotation of heart, interventricular septal defect, etc.

125 mg/kg (6 pups)
-Interventricular septal defect
-Small with brachyury, fused vertebral arches and retarded ossification
-interventricular septal defect and additional digit
-Incomplete inferior vena cava
-Left retinal fold
-Marked malrotation of heart

Control (3 pups)
-Interventricular septal defect
-Diaphragmatic hernia and right azygous vein
-interventricular septal defect

Test substance : Technical biphenyl, purity stated as 99.8% w/w

Conclusion : Biphenyl was clearly fetotoxic and maternally toxic at 1000 mg/kg/day causing mortality of both dams and early-pregnancy loss including complete resorptions. The 500-mg/kg/day dose level was statistically a NOAEL for both dams and fetuses. In spite of the fetotoxicity and maternal toxicity the incidence of malformations was not increased

Reliability : (1) valid without restriction

Modern guideline study under GLP's with clear maternal toxicity achieved
Flag : Critical study for SIDS endpoint
23.02.2005 (46)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT